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Artemisinin Biosynthesis: Developmental and Sugar Regulation of mRNA Levels

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ARTEMISININ BIOSYNTHESIS: DEVELOPMENTAL AND SUGAR REGULATION OF mRNA LEVELS

by

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ABSTRACT

Artemisinin, produced by the plant *Artemisia annua*, is a sesquiterpene anti-malarial therapeutic. Due to the medicinal relevance of this plant product, there is significant interest in understanding how the biosynthetic pathway is regulated at several key steps. The objective of this study is to examine several factors known to influence artemisinin yields to determine if those effects are occurring at the transcriptional level of the biosynthetic pathway.

Artemisinin content has been shown to increase as the plant shifts from vegetative growth to reproductive, flowering growth. To test whether there is a corresponding increase in terpenoid gene expression during the shift to reproductive growth, levels of mRNA of terpenoid genes were measured during flowering budding and full flowering and compared to those measured during vegetative growth. Results indicate that in response to the photoperiod signal to shift to reproductive growth, early cytosolic pathway genes were highly upregulated, while there was no change in early plastidic pathway genes. Late pathway genes specific to artemisinin synthesis were upregulated >6-fold.

Furthermore, glucose has also been shown to stimulate artemisinin production compared to sucrose. To test whether glucose is acting as signal to increase terpenoid gene expression, levels of mRNA of terpenoid genes were measured in glucose- and fructose-treated seedlings and compared to those in sucrose-treated seedlings. Results indicate that in response to treatment with glucose, compared with sucrose, early pathway genes in both compartments were initially upregulated. Transcript levels subsequently

decreased to levels similar to those in sucrose-treated seedlings. ADS was upregulated by glucose, compared with sucrose, reaching a peak at day 7.

Finally, coordinate control of sterol and sesquiterpene synthesis at a critical branch-point in the terpenoid biosynthetic pathway has been demonstrated. To test whether amorpha-4,11-diene synthase (ADS) and squalene sythase (SQS) are coordinately regulated, levels of mRNA of those two genes were measured and compared in both experimental conditions. Results indicate that under the conditions used in this study, ADS and SQS did not show coordinate regulation.

This study was the first to demonstrate that: 1. terpenoid genes relating to artemisinin biosynthesis are regulated at the level of transcript accumulation as the plant shifts from vegetative to reproductive growth; 2. glucose is acting as a signal in artemisinin biosynthesis by upregulating transcript levels for several terpenoid genes.

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Table of Contents

LIST OF FIGURES

LIST OF TABLES

1. INTRODUCTION

1.1. Importance of Artemisinin and its Production in *A. annua*

Artemisinin (Figure 1) is a sesquiterpene lactone secondary metabolite with an endoperoxide bridge produced by the annual herb *Artemisia annua* L. The plant, part of the Asteraceae family, is native to China and has been used there for over two thousand years to treat malaria (Li and Wu, 2003; Hsu, 2006). Artemisinin plays a central role in combating the increasingly drug-resistant, malaria-causing parasite *Plasmodium falciparum*. Artemisinin-based combination therapy (ACT) is currently the most effective means to treat and reduce the transmission rate of malaria (Mutabingwa, 2005; Malenga et al., 2005). In addition to its use in treating malaria, artemisinin has also been demonstrated to be effective against a variety of other diseases, such as hepatitis B (Romero et al. 2005), parasites that cause schistosomiasis (Borrmann et al., 2001), and a range of cancer cell lines (Efferth et al., 2001; Singh and Lai, 2001).

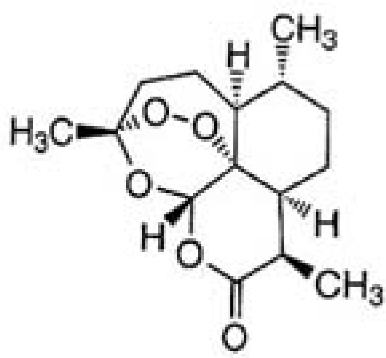


Figure 1. Artemisinin Structure

Artemisinin is synthesized and sequestered primarily in glandular secretory trichomes in *A. annua* (Covello et al., 2007). Although the compound is not observed in the roots of the plant, late pathway genes are expressed in these tissues (Teoh et al., 2006). Production and accumulation of the drug occurs at relatively low levels (0.01-0.8%) and represents a major hurdle in the commercialization of the compound (Abdin et al., 2003). Other avenues of production are being investigated, such as the genetic modification of bacteria and yeast, however the end products of these transgenic pathways are artemisinin precursors, requiring further steps for the complete synthesis of artemisinin (Zeng et al., 2008; Newman et al., 2006; Shiba et al., 2007; Ro et al., 2006).

1.2. Biosynthetic pathway of artemisinin

1.2.1. Early Steps to Isopentenyl Diphosphate in Isoprenoid Biosynthesis

There are two independent, differentially-localized pathways in terpene biosynthesis that converge to yield a common pool of the terpenoid precursor, isopentenyl diphosphate (IPP) (Figure 2) (Lange et al., 2000). The mevalonic pathway (MVA) is located in the cytosol and originates from acetyl-CoA. The key step in this pathway is the conversion of hydroxymethylglutaryl-CoA (HMG) to mevalonate via HMG reductase (HMGR). Several subsequent steps lead to formation of the cytosolic localized pool of IPP.

The other pathway (MEP) to IPP begins with pyruvate and occurs in the plastid with no mevalonate intermediate. The first key step is the synthesis of 1-deoxy-D-xylulose-5-phosphate (DXP) via DXP synthase (DXS). DXP is then converted to 2-C-methyl-D-erythritol-4-phosphate via DXP reductoisomerase (DXR). This is the first committed MEP step towards terpenes. Several subsequent steps yield the plastid pool of IPP. Although IPP present in the cytosol is generally used for the biosynthesis of sterols, sesquiterpenes, triterpenes, and polyterpenes, and the IPP present in the plastid is used for the biosynthesis of monoterpenes, diterpenes and carotenoids, translocation of IPP from the cytosol to the plastid or vice versa may occur depending on the needs of the plant (Lange et al., 2000; Adam et al., 1998).

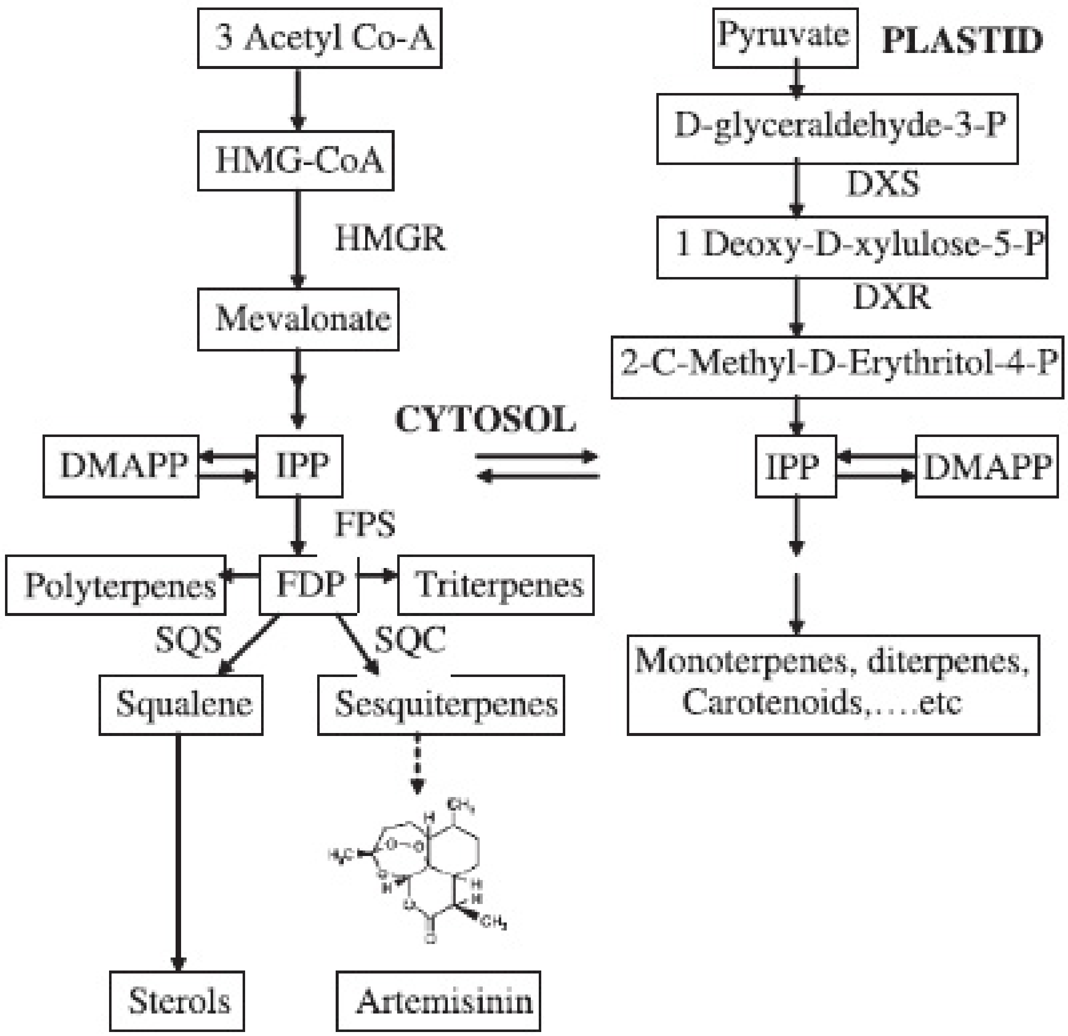


Figure 2. Early biosynthetic pathway for artemisinin: steps in the MVA and MEP pathways and post-IPP steps (taken from Weathers et al., 2006). Abbreviations: HMG-CoA, hydroxymethylglutaryl-CoA; HMGR, HMG-CoA reductase; IPP, isopentenyl diphosphate; DMAPP, dimethylallyl diphophate; FDP, farnesyl diphosphate; FPS, FDP synthase; SQS, squalene synthase; SQC, sesquiterpene cyclase; DXS, 1-deoxy-D-xylulose-5-phosphate synthase; DXR, 1-deoxy-D-xylulose-5-phosphate reductoisomerase.

1.2.2. Cytosolic and Plastidic Metabolic Crosstalk

Evidence exists suggesting that the two compartmentalized pathways of terpene biosynthesis may communicate with each other to regulate metabolic intermediate availability. There are no absolute constraints on the compartmentalization of intermediates in the pathway and the degree of separation probably depends on the species and physiological conditions (Hampel et al., 2005). In *A. annua*, evidence suggests that both the MVA and MEP pathways seem to play a role in artemisinin production. For example, when the cytosolic MVA pathway was disrupted by inhibiting HMGR with mevinolin, artemisinin levels dropped by about 80%. When DXR in the MEP pathway was inhibited by fosmidomycin, artemisinin levels dropped about 70%. Use of both inhibitors resulted in no detectable artemisinin production (Towler and Weathers, 2007).

In Arapidopsis, inhibition of the MVA pathway with the HMGR inhibitor, lovastatin, resulted in an overall decrease in levels of sterols and an increase in levels of carotenoids and chlorophyll (Laule et al., 2003). After the initial drop, levels of sterols recovered to control levels, suggesting redirection of intermediates from the uninhibited MEP pathway. Conversely, inhibition of the MEP pathway with fosmidomycin resulted in an increase in levels of sterols and a decrease in levels of carotenoids and chlorophyll; after 4 days, however, all levels were lower than controls. Patterns of MVA and MEP pathway genes did not correlate with the measured levels of intermediates, suggesting a post-translational mode of regulation.

Supplying plants with exogenous radioactive-labeled carbon substrates enables tracking of metabolic intermediates through specific pathways. Hemmerlin et al. (2003)

showed that inhibiting the MVA pathway resulted in the incorporation of carbon from the MEP pathway into sterols. Inhibition of the MEP pathway, on the other hand, resulted in the incorporation of carbon from the MVA pathway into the plastid isoprenoid, plastoquinone. These results corresponded with a study using an Arabidopsis mutant with increased tolerance to the MVA pathway inhibitor, mevinolin (Rodriguez-Concepcion et al., 2004). In this mutant, resistance to mevinolin occurred due to an upregulation of HMGR. A subset of these mutants was also resistant to the MEP pathway inhibitor, fosmidomycin. The authors concluded that resistance to fosmidomycin was probably due to translocation of intermediates from the cytosol to the plastid to synthesize vital plastid-exclusive compounds. Another study, however, directed carbon flow through either the MEP or MVA pathway through targeted over-expression of the respective genes in those pathways in transgenic tobacco plants (Wu et al., 2006). Analysis of the radio-labeled intermediates, however, demonstrated that little exchange occurred between the two pathways.

The early plastidic pathway gene, DXS, has been shown to be diurnally regulated in correlation with diurnal monoterpene and sesquiterpene emission, suggesting plastidic-derived IPP contributes significantly to the overall pool of IPP (Dudareva et al., 2005). Moreover, this contribution of IPP was suggested to occur in a unidirectional fashion from the plastid to cytosol via a proton symport in the plastid membrane (Dudavera et al., 2005; Hampel et al., 2005). Indeed, isolated chloroplasts have been shown to efficiently transport IPP from the plastid to the cytosol (Bick and Lange, 2003).

1.2.3. Post-IPP Terpene Biosynthesis

Once IPP is formed and available in the cytosol, the next step towards artemisinin biosynthesis is the production of farnesyl diphosphate (FDP) via FDP synthase (FPS). Sequence analysis of FPS from *A. annua* has shown a very close similarity to FPS from other plants (Matsushita et al., 1996). Indeed transgenic *A. annua* plants expressing FPS under a constitutive promoter produced 3-4 times more artemisinin than control lines, suggesting that FPS is one of the regulatory points in artemisinin biosynthesis (Chen et al., 2000). Once FPP is formed, there are a number of different branch points, including to triterpenes, polyterpenes, sterols, and sesquiterpenes (Figure 2).

There is evidence suggesting that in plants the option to branch towards either sterols or sesquiterpenes is under coordinate control. To produce sesquiterpenes, a sesquiterpene cyclase (SQC) is the required first catalyst; to produce sterols, the required first catalyst is a specific squalene synthase (SQS). In plants, when sterol production is upregulated, sesquiterpene production is often downregulated, and vice versa. Vogeli and Chappell (1988) demonstrated that introducing fungal elicitors to tobacco cell culture suspensions caused a rapid increase in sesquiterpenoid production paralleled by a rapid decrease in sterol production. This coordinate control of SQS and SQC genes has also been demonstrated in potato tubers (Yoshioka et al., 1999; Krits et al., 2007). Upon wounding, metabolic flow is directed towards sterols by the upregulation of SQS and downregulation of SQC. On the other hand, exposure of the wound to fungal pathogens or elicitors causes redirection of metabolic flow towards sesquiterpenoid phytoalexins and SQC is upregulated while SQS is downregulated. In *A. annua*, Towler and Weathers (2007) showed that coordinate control of these two pathways may also be in play.

Inhibition of SQS with miconazole caused a significant increase in artemisinin, suggesting carbon was channeled towards sesquiterpene synthesis once sterol biosynthesis was inhibited.

1.2.4. Post-FDP Artemisinin Biosynthesis

A variety of sesquiterpene cyclases exist in *A. annua*, including epi-cedrol synthase, beta-caryophyllene synthase, (E)-beta-farnesene synthase, germacrene A synthase, and an uncharacterized SQC (Figure 3) (Weathers et al., 2006). Production of the first specific precursor of artemisinin, however, involves the formation of amorpha-4,11-diene via the sesquiterpene cyclase, amorpha-4,11-diene synthase (ADS) (Figure 4) (Bouwmeester et al., 1999; Kim et al., 2008; Kim et al., 2006; Wallaart et al., 2001).

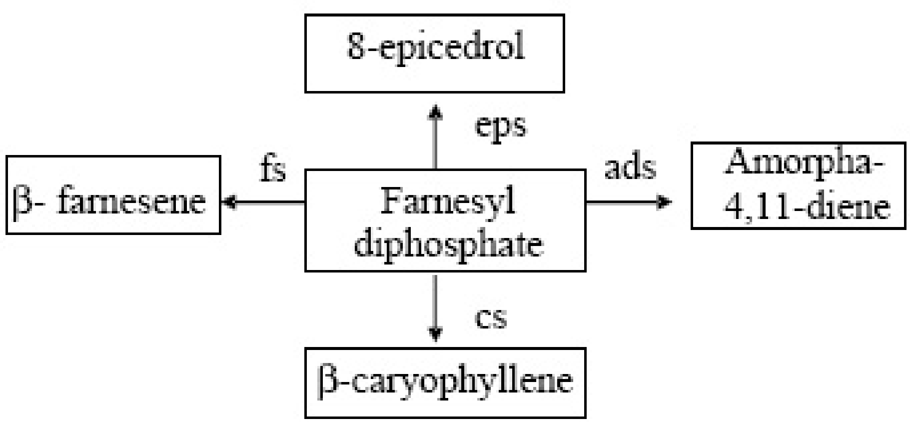


Figure 3. Sesuiterpene cyclases isolated from *A. annua* (taken from Weathers et al. 2006). Abbreviations: eps, epi-cedrol synthase; cs, beta-caryophyllene synthase; fs, (E)-beta-farnesene synthase.

The final steps of the biosynthesis of the artemisinin-precursor, dihydroartemisinic acid, have not been fully elucidated, however they may involve up to three reactions that have been shown to be catalyzed *in vitro* by the cytochrome P450, CYP71AV1 (Figure 4) (Teoh et al., 2006). The first reaction is the hydroxylation of amorpha-4,11-diene to form artemisinic alcohol, followed by the oxidation of the alcohol to artemisinic aldehyde.

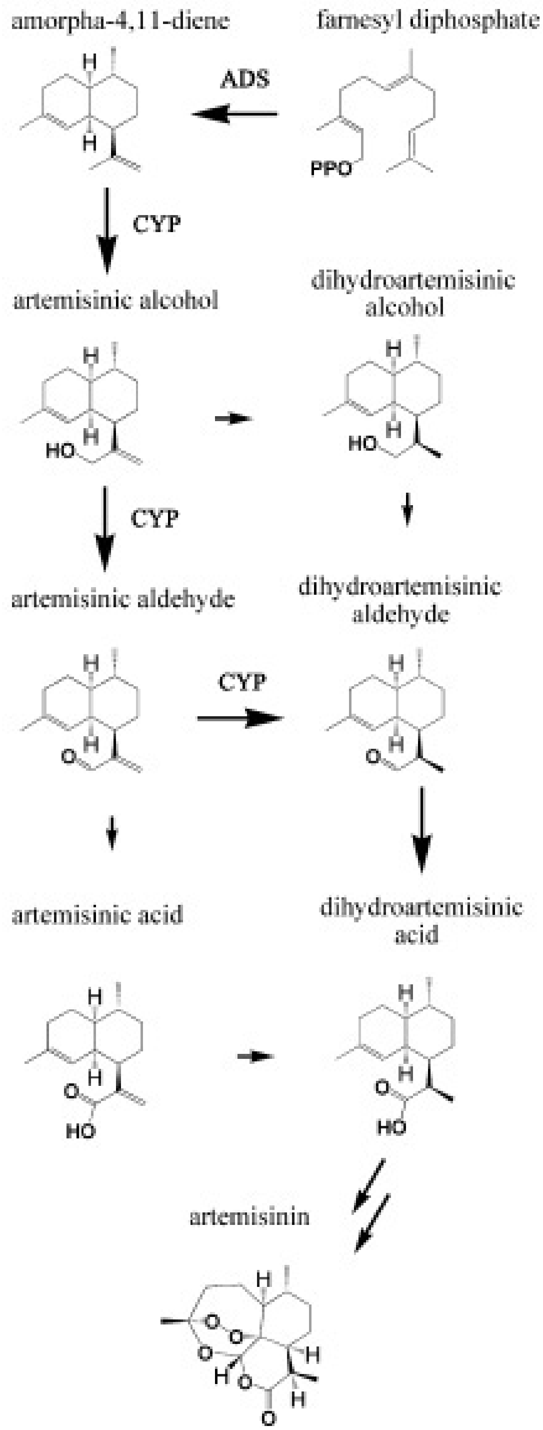


Figure 4. Late biosynthetic pathway of artemisinin: post-FDP steps leading towards artemisinin production (taken from Teoh et al. 2006). Abbreviations: ADS, amorpha-4,11-diene synthase; CYP, cytochrome P450 CYP71AV1.

Artemisinic aldehyde is then reduced to dihydroartemisinic aldehyde and a final oxidation converts the aldehyde to the acid form (Wallaart et al., 1999a). Although CYP71AV1 has the capability to catalyze the aforementioned series of reactions, there may be several other as-yet unidentified enzymes including an alcohol dehydrogenase, an aldehyde hydrogenase, and an aldehyde dehydrogenase to catalyze *in vivo* the oxidation, reduction, and second oxidation, respectively (Figure 5) (Bertea et al., 2005). Some controversy exists in artemisinin biosynthesis because other precursors have also been identified, such as dihydroartemisinic alcohol and artemisinic acid. These results have led some to suggest the existence of multiple chemotypes and indeed the presence and relative levels of intermediates differs by the plant's geographic location (Wallaart et al., 2000).

The conversion of dihydroartemisinic acid (DHAA) to artemisinin is thought to be a non-enzymatic, photo-oxidative reaction through the intermediate dihydroartemisinic acid peroxide (DHAA peroxide). This conclusion is based on several observations by Wallaart et al. (1999b, 2000) The synthesis of DHAA peroxide from DHAA occurs exactly like the photo-oxidation of polyunsaturated fatty acids to lipid hydroperoxides, and DHAA peroxide has been observed in *A. annua* tissues. Furthermore, DHAA peroxide has been shown to rapidly convert to artemisinin through air oxidation. This mechanism of synthesis would support a possible physiological role of artemisinin, acting as a sink for reactive oxygen species in stress conditions.

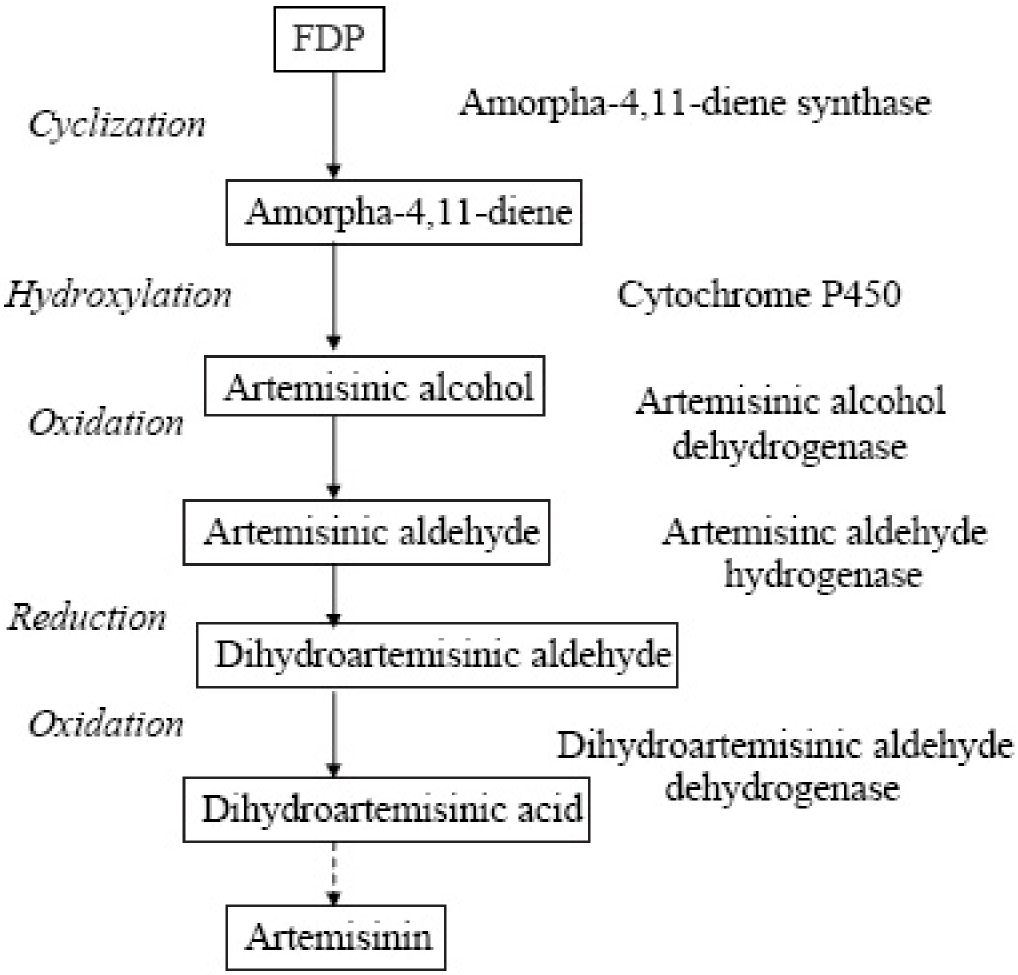


Figure 5. Intermediates and enzymes in the late biosynthetic pathway of artemisinin production (taken from Bertea et al. 2005). Abbreviations: FDP, farnesyl diphoshate.

1.3. Regulation of the Artemisinin Biosynthetic Pathway

Although many of the genes involved in artemisinin biosynthesis have been isolated and cloned from *A. annua*, little is known about their regulation. Most of the data relates to the effects of light, culture age, and tissue location on the expression of these genes with most results to date measured in hairy root cultures (Souret et al., 2003; Teoh et al., 2006).

1.3.1. Regulation and Developmental Stage

The shift from vegetative growth into reproductive growth is another factor that has been shown to influence artemisinin production. Artemisinin yields were reported as being 4- to 11-fold higher in flowers than in leaves (Ferreira et al., 1995). Since many terpenoids are floral fragrances, up-regulation of terpene biosynthesis during the shift from vegetative to flowering is not surprising. Previous studies had suggested that a link between flowering and artemisinin biosynthesis exists, although these studies differed in relation to the stage during which peak artemisinin production was reached. One study, for example, showed that peak artemisinin production occurs in the budding stage just before flowering (Liersch et al., 1986; Chan et al., 1995; Woerdenbag et al., 1994), while others reported that peak production was only reached when the flowers were in full bloom (Ferreira et al., 1995). Some genes related to terpene synthesis in other plants have also been shown to be transcriptionally activated as the shift to flowering occurs (Dudareva et al., 2003). More recently, two separate studies investigated the link between flowering and artemisinin. The flowering promoter factor gene from Arabidopsis, fpf1, and the early flowering gene from Arabidopsis, CONSTANS, were constitutively expressed in *A. annua*, and although flowering was induced approximately two to three weeks earlier in transgenic lines, there was no corresponding increase in artemisinin biosynthesis. These data suggested that there was no direct regulatory link between flowering and artemisinin synthesis, and that some other factor is likely contributing to the observed increase in artemisinin content as the shift to the reproductive stage progresses (Wang et al., 2004; Wang et al., 2007).

1.3.2. Do Sugars Regulate Terpene Metabolism?

Disaccharide- and monosaccharide-derived carbon sources are additional factors that have been shown to differentially affect artemisinin production (Weathers et al., 2004, Wang and Weathers, 2007). When *A. annua* seedlings were grown on equimolar amounts of sucrose, glucose or fructose in terms of carbon, artemisinin yields differed depending on the sugar. For example, artemisinin levels increased significantly in seedlings grown on glucose relative to those grown on sucrose, while levels decreased significantly when seedlings were grown on fructose relative to those grown on sucrose. Furthermore, the levels of artemisinin increased in direct proportion to the increasing ratio of glucose to fructose (Wang and Weathers, 2007). These results suggested that besides acting as carbon sources, sugars may be involved in also regulating artemisinin production.

The effect of sucrose on anthocyanins has been studied in cell culture suspensions of *Vitis vinifera* (Vitrac et al., 2000). Levels of anthocyanins were shown to increase in the presence of sucrose and it was suggested that this increase was due to signal transduction mediated by hexokinase. Those results were obtained using three glucose analogs. Mannose, a glucose analog that is efficiently transported and phosphorylated by hexokinase, was able to induce an increase in anthocyanins, while 3-*O*-methylglucose, another glucose analog that is transported but not efficiently phosphorylated by hexokinase, did not yield an increase. An inhibitor of hexokinase, mannoheptulose, was unable to cause an increase in anthocyanins in the presence of sucrose.

1.3.3. Sugars Are Sensed as Signals

Sugar sensing plays an important role *in vivo* in modulating a wide range of responses, including stress response (starvation), growth (seed germination, hypocotyl elongation), development (flowering and senescence), and metabolism (Rolland et al., 2006). Sugar sensing is the mechanism by which sugars take on a signaling role, interacting with a sensor molecule to initiate a signal cascade to cause cellular responses and changes in gene expression (Smeekens, 2000; Rolland et al., 2006; Gibson, 2005). Since sugars are also used in carbon and energy metabolism, it is challenging to differentiate any perceived cellular responses related to sugar sensing from those related to global energy state or metabolic intermediate feed-back or feed-forward mechanisms.

Sugar sensing has been studied extensively in yeast which prefer fermenting sugars to ethanol for energy. One of the major sugar sensing pathways for that effect is the glucose repression pathway. Genes responsible for respiration, gluconeogenesis, uptake/metabolism of alternate carbon sources are transcriptionally repressed in the presence of glucose (Rolland et al., 2001; Rolland et al., 2002a). The process is more complicated in plants, because they have differentiated tissues (roots, stems, leaves, etc.) and they use source-sink activities to maintain their energy balance (Roitsch et al., 1999). Furthermore, this balance is diurnally shifted. During the day, leaves are source tissues where glucose is synthesized, while roots are sink tissues where glucose is stored as starch. During the night, glucose is liberated from starch and transported to shoot tissues as a component of sucrose. There it is metabolized for energy. Since the concentration of sugars will determine if a location is a source or sink, the cell needs to be able to sense the level of different sugars to coordinate source-sink related regulation (Roitsch et al., 1999).

A number of studies have provided evidence for sugar sensing in Arabidopsis and other plants (Thum et al., 2004; Price et al., 2004; Gonzali et al., 2006). In Arabidopsis, sugar sensing and regulation of growth and development has been studied by characterizing mutants which are sugar hypersensitive or insensitive (Rolland et al., 2002b). The mutant alleles are often genes involved in hormone and stress pathways. Hexokinase in particular appears to play a central role as a sugar sensor that mediates signaling separate from its catalytic ability (Rolland et al., 2001; Rolland et al., 2002b; Moore et al., 2003; Yanagisawa et al., 2003). This uncoupled feature of signaling and catalytic abilities is illustrated by the ability of HXK1 constructs that lack ATP binding or phosphoryl transfer ability to enable the rescue of the wild-type phenotype in a glucose insensitive, *gin2 atHXK1*, mutant. Further evidence of gene regulation initiated by sugar-sensing of glucose by hexokinase can be observed by the ability of hexokinase to translocate into the nucleus where it is found as part of a high molecular weight complex, suggesting a direct role in transcription-level regulation (Moreno et al., 2005; Palomino et al., 2005; Yanagisawa et al., 2003).

Sugars may also be sensed at the cell surface. A mutant in the surface receptor RGS1, which interacts with the alpha-subunit of G-protein coupled receptors, displays insensitivity to a high glucose concentration, while displaying hypersensitivity when over-expressed (Chen and Jones, 2004). Another potential sensor is analogous to sucrose transporter-like sensors in yeast. In Arabidopsis, they are homologous to sucrose transporters, but do not have detectable transporting activity (Barker et al., 2000). These are thought to play a role in sugar sensing. Using microarray technology, collections of genes from Arabidopsis were identified in sugar starvation conditions that were up or downregulated. By using bioinformatic approaches, several common cis-regulatory elements were identified in the promoter regions of these genes, including the sucrose-responsive element (SURE), A- B- and G-boxes, and an SP8-motif (Maeo et al., 2001; Acevedo-Hernadez et al., 2005; Kim et al., 2004; Lu et al., 2002; Masaki et al., 2005a; Masaki et al., 2005b; Morikami et al., 2005; Sun et al., 2003; Tsukagoshi et al., 2005).

Sugar sensing also involves protein kinases, which are important mediators of signal cascades. One such protein kinase that is encoded by the gene, sucrose non-fermenting 1 (*Snf1*), has a homolog in plants called Snf1 related- protein kinase (*SnRK*) that is thought to be involved in metabolic regulation and sugar starvation response (Halford et al., 2003). SnRK is activated by sucrose and inactivates several enzymes such as HMGR and sucrose phosphate synthase *in vitro* through phosphorylation (Sugden et al., 1999). The regulation of HMGR in particular is of interest since this enzyme is an early key enzyme involved in the MVA pathway of terpenoid synthesis.

2. Objectives and Hypotheses

A significant bottleneck exists in the production of sufficient yields of artemisinin for treatment of malaria and other diseases. Clearly, a deeper understanding of the biosynthetic pathway of artemisinin and its regulation is required in order to maximize the potential for developing artemisinin production systems. The overall objective of this study is to examine several factors known to influence artemisinin yields to determine if those effects are occurring at the transcriptional level of the biosynthetic genetic pathway.

The steady state mRNA levels of key genes in the artemisinin pathway under various experimental conditions were measured using real-time PCR.

This research tests the following hypotheses: 1. ADS expression would be up-regulated and SQS would be down-regulated if carbon is coordinately channeled away from sterols and into sesquiterpenes; 2. an increase in terpenoid gene expression would occur with a shift from vegetative to reproductive growth if flowering correlates with increased artemisinin production; 3. in response to glucose, one or more genes in the pathway will be upregulated compared to either sucrose or fructose if glucose acts as a signal in controlling terpene biosynthesis.

The specific objective for the first hypothesis involves measuring relative levels of mRNA for SQS and ADS under the above experimental conditions to provide evidence that carbon channeling away from sterols is occurring. The specific objective for the second hypothesis involves measuring relative levels of mRNA of the early (DXS, DXR, HMGR, and FPS) and late (ADS and CYP71AV1) genes in artemisinin biosynthesis in *A. annua* as seedlings are shifted from vegetative growth to flowering. The specific objective for the final hypothesis is to compare the relative levels of mRNA of the early (DXS, DXR, HMGR, and FPS) and late (ADS and CYP71AV1) genes in artemisinin biosynthesis in *A. annua* seedlings grown separately in three sugars: sucrose, glucose, or fructose.

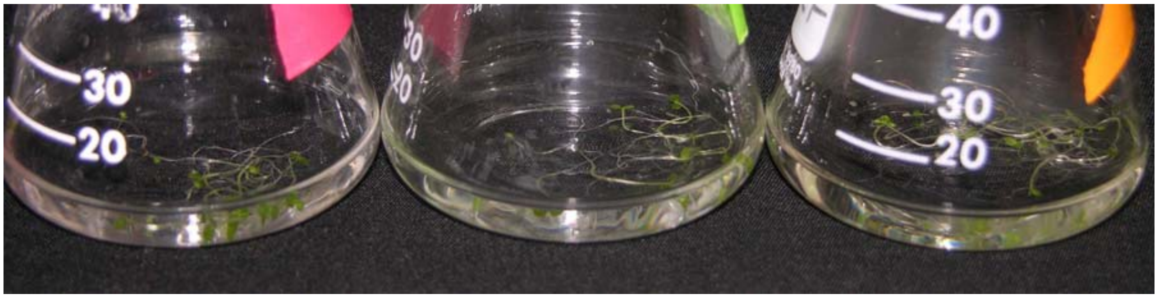
3. METHODS AND MATERIALS

3.1. Growth conditions

Seeds from the YU strain (Weathers et al., 1994) of *Artemisia annua* L. were used in all experiments. For experiments that provided individual sugars to plants, seeds were surface-sterilized and synchronously germinated according to the protocol developed by Wang and Weathers (2007). Seeds were washed with 10% (v/v) bleach for 12 minutes followed by 70% (v/v) ethanol for 5 minutes, then washed for 5 minutes three consecutive times with 0.1% (v/v) sterile PPM (Preservative for Plant Tissue Culture Media, Plant Cell Technology, Inc.). After the third wash, the seeds were immersed in 0.1% sterile PPM in a 125 ml Erlenmeyer flask and incubated in the dark at 4 °C. After 3 days, the PPM was replaced with sterile Gamborg's B5 medium (Gamborg et al., 1968) with 3% (w/v) sucrose at pH 5.7 and the seeds were further incubated in the dark at 4 °C for an additional 3 days. The seeds were then moved to a rotary shaker at 140 rpm under continuous cool-white fluorescent light (20-30 μmol m^{-2} s^{-1}) and incubated for 5 more days to germinate and develop to the two-cotyledon stage. The seedlings were then washed 7 times with sterile sugar-free B5 medium at pH 5.7 to remove exogenous sugars and their physiological effects after which they were immersed in sugar-free B5 medium and incubated 1 more day in the dark at 4 °C. The seedlings were then inoculated in experimental media according to the protocol developed by Towler and Weathers (2007). Ten seedlings were placed in a 50 ml Erlenmeyer flask containing 5 ml autoclaved B5 medium at pH 5.7 to which filter-sterilized 3% (w/v) sucrose, glucose, or fructose was added (Figure 6A-D). The flasks were placed on a rotary shaker at 100 rpm under continuous cool-white fluorescent light. Samples were harvested after 1, 2, 3, 4, 7, and 14 days of incubation. Three replicates were harvested at each time point for each sugar treatment.

A. annua plants were also grown to obtain vegetative or reproductive (flowering) plants. Seeds were evenly spaced and germinated in soil flats under a 16-hour photoperiod using cool-white fluorescent light. Plantlets were grown under a 16-hour vegetative photo-period for 2 weeks, after which half of the plants were induced to flower precociously by switching them to an 8-hour photo-period and grown for a total of 4-6 weeks. The other half were kept under a vegetative (16-h) photo-period for 4 weeks and then harvested (Figure 7A). Reproductive plants were harvested at two stages: initial flower budding (Figure 7B) and full flowering (Figure 7C). Plants were harvested and separated into shoots and roots. Leaves and flowers or flower buds from each stage of the flowering plants were removed from the central stem and homogenized together.

A.



B.

C.

D.

Figure 6. *A. annua* plantlets exposed to sucrose (red), glucose (green), or fructose (orange). A, 0 days; B, 2 days; C, 7 days; D, 14 days.

A.

B.

C.

Figure 7. *A. annua* during the vegetative and reproductive growth stages. A, vegetative plant; B, plant after bolting and at formation of flower buds; C, plant in full flower.

3.2. RNA Extraction and Transcript Analysis

Immediately after harvest, plant samples were flash frozen in liquid nitrogen and ground into a fine powder using a chilled mortar and pestle. These ground samples were kept in a -80 °C freezer for further analysis. For RNA isolation, 50-100 mg of ground plant tissue was homogenized with 1 ml TRIzol reagent (Invitrogen, Carlsbad, CA, cat. # 15596-018). The solution was incubated for 15 minutes at room temperature, after which 200 µl of chloroform was added. The solution was shaken vigorously and incubated for 15 minutes at room temperature. The cellular debris was removed by centrifuging at 12,000 x g for 10 minutes. The aqueous phase was transferred to a fresh 1.5 ml Eppendorf tube and mixed with 0.5 ml isopropanol. RNA was allowed to precipitate out of solution for 10 minutes and then centrifuged at max speed for 10 minutes. The pellet was washed briefly with 1 ml 75% ethanol and then allowed to air-dry. After 5 minutes, the pellet was resuspended in DEPC-treated water and the concentration of RNA was quantified at 260 nm.

To remove contaminating genomic DNA from the RNA sample, the Turbo DNA-free kit (Ambion, Austin, TX, cat. # AM1907) was used according to the manufacturer's specifications. A maximum of 10 ug of nucleic acid was added to 50 µl DNase reaction, containing 1X DNase buffer, DEPC-treated water, and 4 U DNase. The reaction was incubated at 37 °C for 30 minutes, after which an additional 4 U of DNase was added. The reaction was then incubated for another 30 minutes at 37 °C.

The RNA transcripts were reverse-transcribed into cDNA using the DyNAmo cDNA synthesis kit (New England Biolabs, Ipswich, MA, cat. # F-470L) following the manufacturer's specifications. Random hexamers were used to randomly prime the RNA for cDNA synthesis instead of oligo-dT primers in order to concurrently reverse-transcribe the 18S rRNA transcripts with the mRNA. The reverse transcription reaction was incubated at 37 °C for 1 hour, and aliquots were added directly to subsequent PCR reactions.

Primers were designed for the eight genes using PrimerSelect (Lasergene, DNAStar, Inc) based on cDNA sequences specific for *Artemisia annua* available at NCBI and are listed in Table 1. Primer pairs were designed to have similar melting temperatures and to amplify 200-300 bp fragments. PCR was performed with each primer pair to ensure sufficient and specific amplification.

Table 1. Primer sequences for target gene amplification by RT-PCR.

Gene	Direction	Sequence (5' => 3')	Base Pairs	Product Length
ADS	Forward	ATACAACGGGCACTAAAGCAACC	23	297 bp
ADS	Reverse	GAAAACTCTAGCCCGGGAATACTG	24	297 bp
CYP	Forward	GGGGTTAGGGATTTAGCCAGAA	22	218 bp
CYP	Reverse	AATTGCCTCCAGTACTCACCATAA	24	218 bp
DXR	Forward	ATTGCTGGCGGTCCCTTTGTTCTT	24	237 bp
DXR	Reverse	CTTTTCTCCCCATGCTCAGTTAGG	24	237 bp
DXS	Forward	ATGGGTTGGCGGGATTCAC	19	274 bp
DXS	Reverse	CCGTCAAGATTGGCAGTAGGTAAA	24	274 bp
FPS	Forward	GTATGATTGCTGCGAACGATGGA	23	211 bp
FPS	Reverse	CGGCGGTGAATAGACAATGAATAC	24	211 bp
HMGR	Forward	GGTCAGGATCCGGCCCAAAACATT	24	251 bp
HMGR	Reverse	CCAGCCAACACCGAACCAGCAACT	24	251 bp
SQS	Forward	GTTCTTCGCGCTCTTGATACTG	22	208 bp
SQS	Reverse	CAATTGCCTCCTGATAACCTCTC	23	208 bp
18S	Forward	TCCGCCGGCACCTTATGAGAAATC	24	219 bp
18S	Reverse	CTAAGAACGGCCATGCACCACCAC	24	219 bp

Abbreviations: ADS, amorpha-4,11-diene synthase; CYP, P450 CYP71AV1; DXR, 1-deoxy-D-xylulose-5-phosphate reductoisomerase; DXS, deoxy-D-xylulose-5-phosphate synthase; FPS, farnesyl disphosphate synthase; HMGR, 3-hydroxy-3-methylglutaryl-CoA reductase; SQS, squalene synthase; 18S, 18S ribosomal small subunit.

Real-time PCR was performed using the Bio-Rad iCycler Real-time PCR system. Reagents used for real-time PCR were supplied as part of the iQ SYBR Green Supermix (Bio-Rad, Hercules, CA, cat. #170-888) according to the manufacturer's specifications. The protocol used was a three-step amplification followed by a melt-curve analysis. For each amplification cycle, there was a denaturation step at 94 °C, an annealing step at 53 °C, and an extension step at 72 °C. Thirty-five cycles were used. Relative fold changes in gene expression were calculated based on the $2^{-\Delta\Delta CT}$ comparative method (Livak and Schmittgen, 2000; Sehringer et al., 2005; Cikos et al., 2007). In this method, levels of target gene amplification in an experimental sample are compared to levels of target gene amplification in another sample or standard, both of which are first normalized to levels of amplification of a normalizing gene. This gene should be highly, stably, and constitutively expressed in all conditions and tissues that are to be analyzed (Deprez et al., 2002; Thellin et al., 1999; Schmittgen et al., 2000; Brunner et al., 2004). For this work, the 18S ribosomal small subunit gene was used as a normalizing factor. Normalized levels of target gene amplification in glucose- and fructose-treated plantlets were expressed as fold changes of target gene expression relative to normalized levels of target gene amplification in sucrose-treated plantlets for the sugar experiments. For the developmental experiments, normalized levels of target gene amplification in flowering and budding plants were expressed as fold changes of target gene expression relative to normalized levels of target gene amplification in vegetative plants.

3.3. Statistical Analyses

All experiments were run in triplicate for the sugar treatment experiments, and quintiplicate for the developmental stage experiments. Fold change values were expressed as the mean ± SD. Results were averaged and compared against controls to determine statistical differences. Statistical analyses were performed using the Mann-Whitney *U* test (Yuan et al., 2006). Statistically significant results are those with a P-value less than or equal to 0.05. Real-time PCR data and calculated fold change values and standard deviations for the carbon source experiments are listed in Tables A1 (glucose and fructose treatments at each day relative to sucrose treatments) and A2 (all treatments at each day relative pre-treatment at day 0) in Appendix 1. Real-time PCR data and calculated fold change values and standard deviations for the developmental stage experiments are listed in Table A3. P-values indicating statistical significance of gene expression in glucose and fructose treatments compared to sucrose treatment are listed in Tables A4 and A5 (Appendix 1).

4. RESULTS

4.1. Developmental Regulation of Artemisinin Biosynthesis

Artemisinin has been shown to accumulate to higher levels as the plant shifts to a reproductive, flowering phase (Ferreira et al., 1995). The timing of this accumulation is contested and reports differ as to whether higher levels occur in budding (Liersch et al., 1986; Chan et al., 1995; Woerdenbag et al., 1994) or flowering plants (Ferreira et al., 1995). Although flowering does not directly influence artemisinin production, there is clearly a correlation between the shift to a reproductive phase and production of artemisinin. This study measured the levels of artemisinin biosynthetic gene transcripts in the plant at three distinct developmental stages: vegetative growth, initial flower bud formation, and full flowering.

Compared to plants grown vegetatively, there was a striking response in the cytosolic pathway genes HMGR and FPS (Figure 8); HMGR transcript levels in budding plants increased >1,000 fold, while FPS transcript levels increased over 400 fold. For both genes, transcript levels were significantly lower in plants at full flowering, however these levels were still >100 fold greater than those in vegetative plants.

In contrast to the large increase in transcripts observed for HMGR and FPS, response of the other terpenoid genes was less dramatic. Levels of gene transcripts specific to artemisinin biosynthesis (ADS and CYP71AV1) were increased about 6 fold when the plant was in the budding phase compared to the vegetative phase (Figure 9). At full flower, however, transcription of both genes dropped to levels at or below that of vegetative plants (Figure 9). Interestingly, neither of the plastid localized genes, DXR and DXS, differed from those in the vegetative stage, and transcript levels for both genes were significantly lower when the plant was in full flowering (Figure 9). SQS transcription also was not significantly different at any developmental stage (Figure 9).

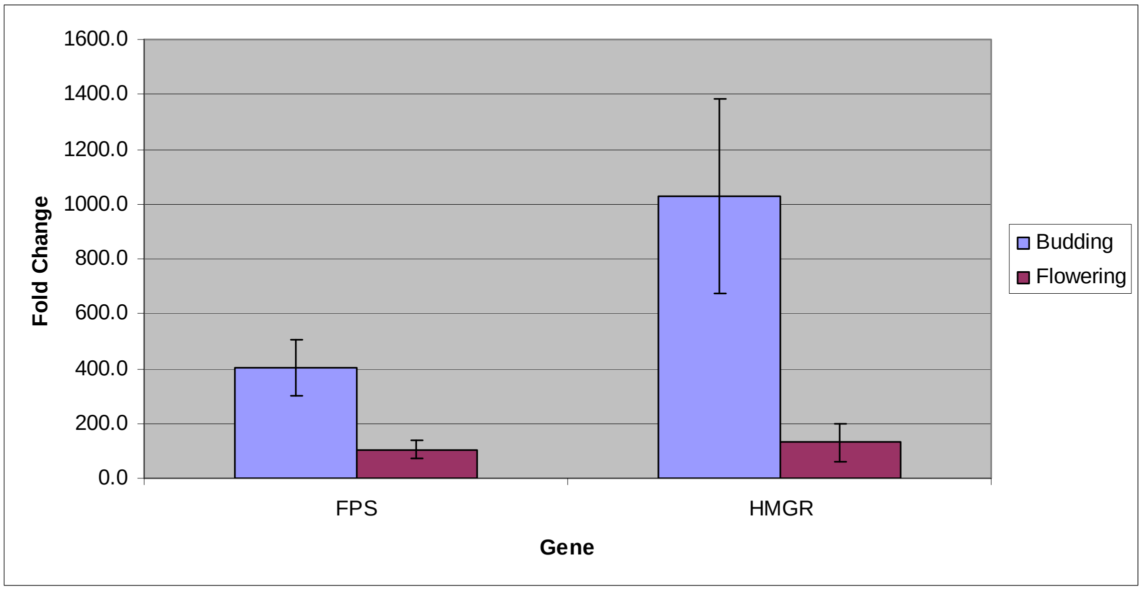


Figure 8. Levels of FPS and HMGR gene expression in budding and flowering plants compared to vegetative plants. Vegetative levels = 1.0. HMGR is the first committed step in the cytosolic terpenoid pathway and FPS is the first committed step towards terpenes and sterols in the cytosol. Abbreviations: FPS, farnesyl diphosphate synthase; HMGR, hydroxymethylglutaryl-CoA reductase.

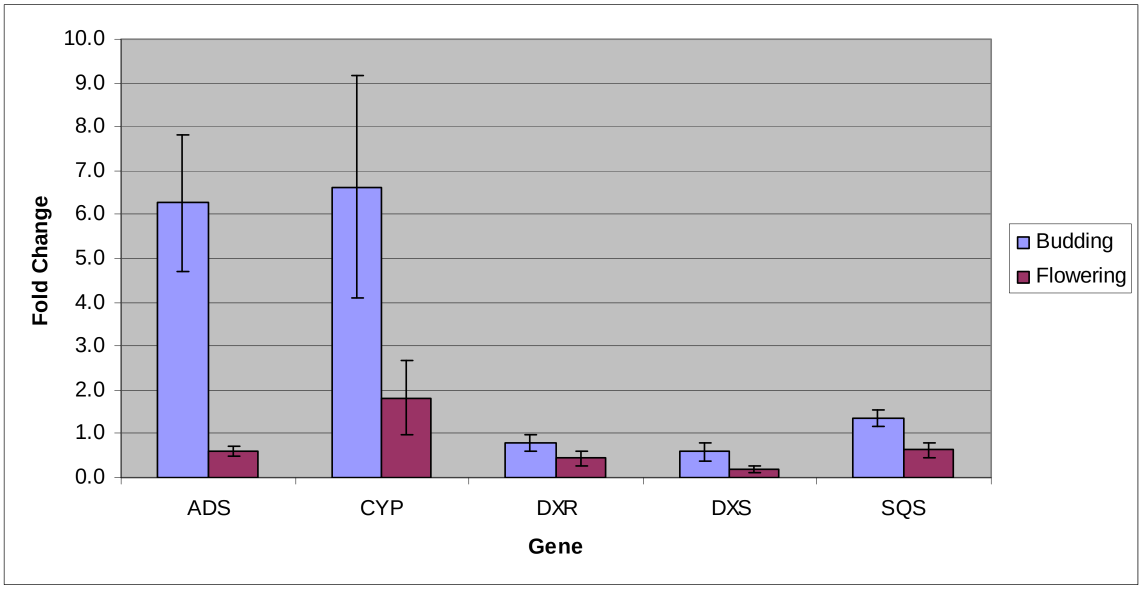


Figure 9. Levels of gene expression in budding and flowering plants compared to vegetative plants. Vegetative levels = 1.0. ADS and CYP are specific genes for artemisinin biosynthesis. DXR and DXS are key steps in the early plastidic biosynthetic pathway. SQS is the first committed steps towards sterols. Abbreviations: ADS, amorpha-4,11-diene synthase; CYP, cytochrome P450 CYP71AV1; DXR, 1-deoxy-D-xylulose-5-phosphase reductoisomerase; DXS, 1-deoxy-D-xylulose-5-phosphate synthase; SQS, squalene synthase.

4.2. Sugar Regulation of Artemisinin Biosythensis

Although both mono- and disaccharides are known to act as both carbon sources and signals in plants (Smeekens, 2000; Rolland et al., 2006; Gibson, 2005), prior work with *A. annua* showed that glucose in particular played a key role in artemisinin biosynthesis (Weathers et al., 2004; Wang and Weathers, 2007). This study, therefore, compared the effect of glucose and fructose to that of sucrose with respect to regulation of artemisinin biosynthetic genes at the level of mRNA transcription.

Because of the stimulatory effect of glucose on artemisinin production (Wang and Weathers, 2007), the first two genes studied were ADS and CYP. ADS mRNA levels in seedlings in all three sugar treatments was significantly greater than those of pre-treated seedlings by day 2 (Figure 10A). Levels of mRNA accumulation continued to increase during days 3 and 4 in sucrose- and glucose-treated seedlings, after which levels reached a plateau during days 7 and 14, whereas ADS mRNA levels in fructose-treated seedlings continued to increase through the duration of the experiment, but still were lower than either sucrose- or glucose-fed seedlings.

Although expression of ADS initially decreased approximately 50% in glucose-grown seedlings relative to sucrose, expression steadily increased and was significantly upregulated by day 3 (Figure 10B). Relative levels of ADS transcripts reached a peak at day 7, when ADS was expressed at almost 4 times that of sucrose-grown seedlings. Although the levels seemed to decline slightly by day 14, the results were not statistically different. In contrast to results in glucose, relative ADS expression in fructose-grown seedlings was consistently lower through day 7, compared to sucrose-grown seedlings. Although levels of ADS expression appeared to increase at days 7 and 14, compared to that of seedlings in sucrose, differences were not significant.

A.

B.

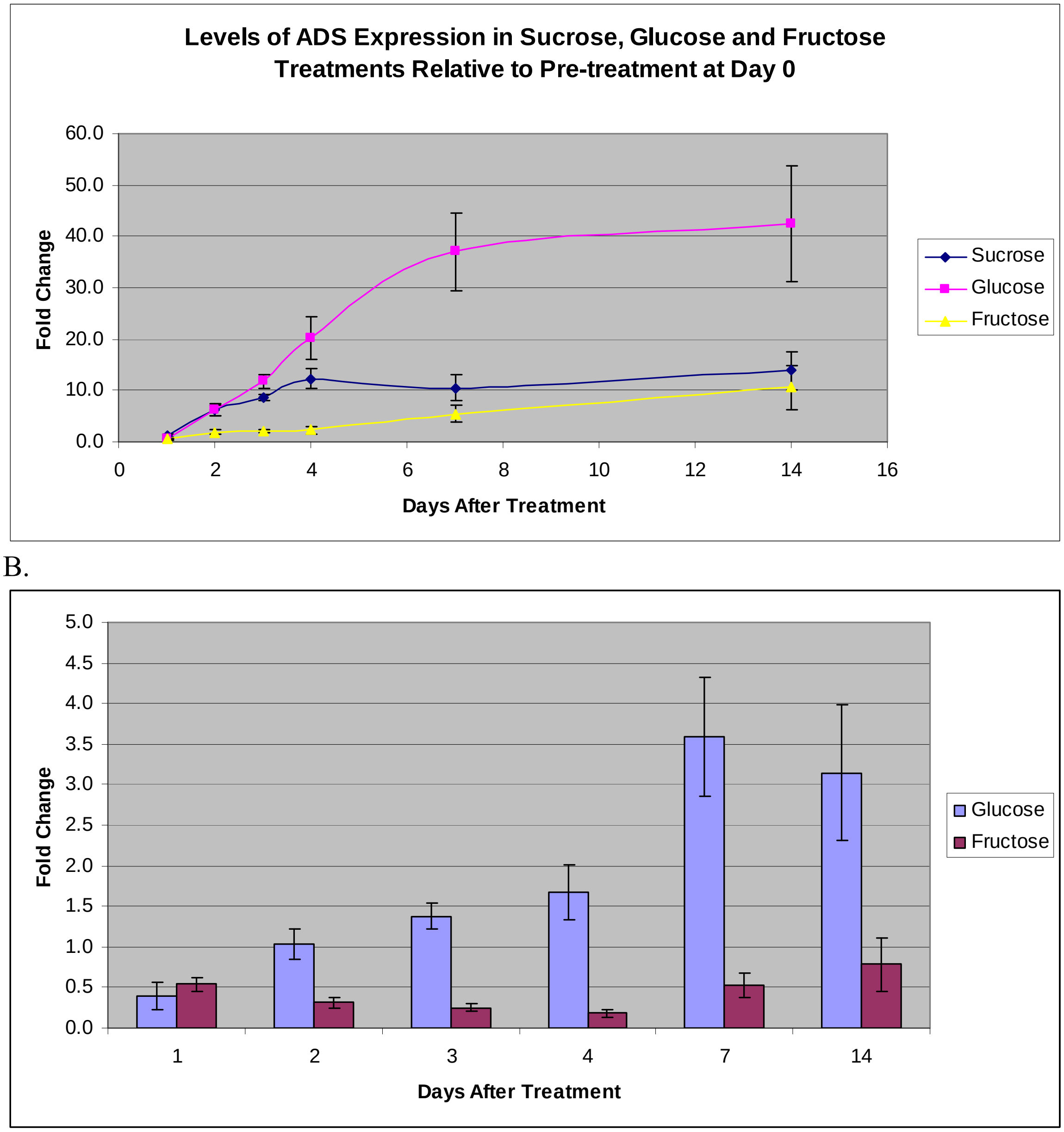
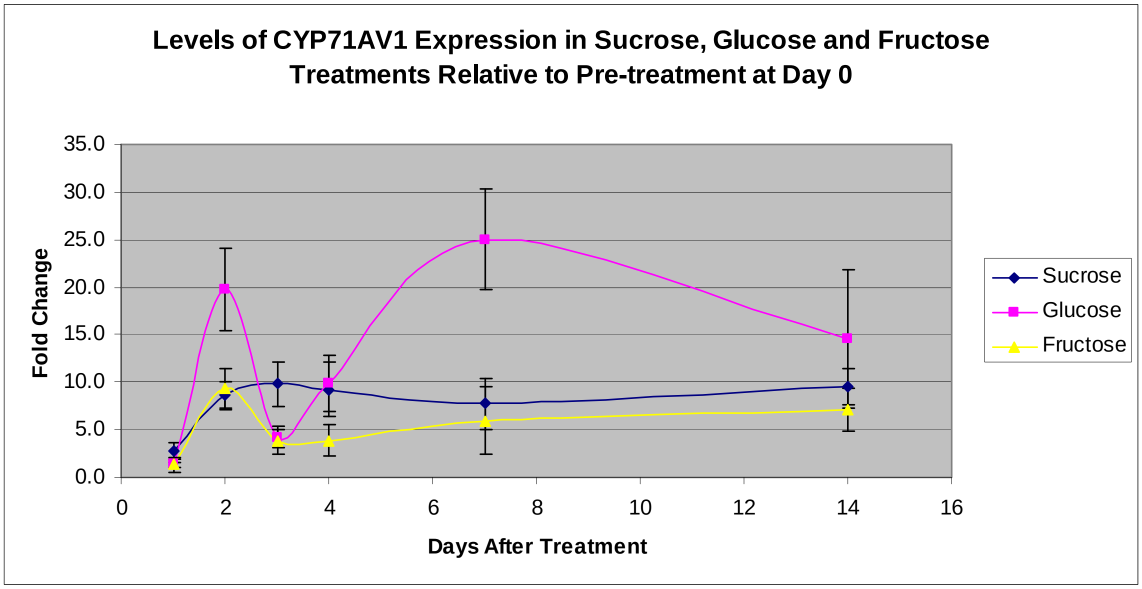


Figure 10. A, Levels of ADS expression in sucrose, glucose and fructose treatments relative to pre-treatment at day 0. Day 0 level = 1.0; B, Levels of ADS expression in glucose and fructose treatments relative to sucrose treatments. Sucrose level = 1.0.

Levels of CYP71AV1 mRNA in all three sugar treatments compared to pre-treatment at day 0 was significantly greater at day 2, and remained so for the duration of the experiment (Figure 11A). Levels of CYP71AV1 mRNA in glucose- and fructose-treated seedlings dropped at day 3, unlike those in sucrose-treated seedlings. This drop was especially pronounced in the glucose-fed seedlings (Figure 11A). Levels for all three sugars, however, were not statistically different at day 14. When compared to ADS, CYP71AV1 showed a different pattern of expression in both glucose and fructose treatments (Figure 11B). After 2 and 7 days post-inoculation into glucose, CYP71AV1 mRNA accumulated to about 2 and 3 times that of seedlings grown in glucose, respectively. Similar to the response of ADS, fructose appeared to downregulate CYP71AV1 relative to sucrose. In contract to ADS, however, the response of CYP71AV1 was biphasic; its expression appeared to fluctuate up and down (Figure 11B, compare days 2 and 7 to days 3 and 14).

A.



B.

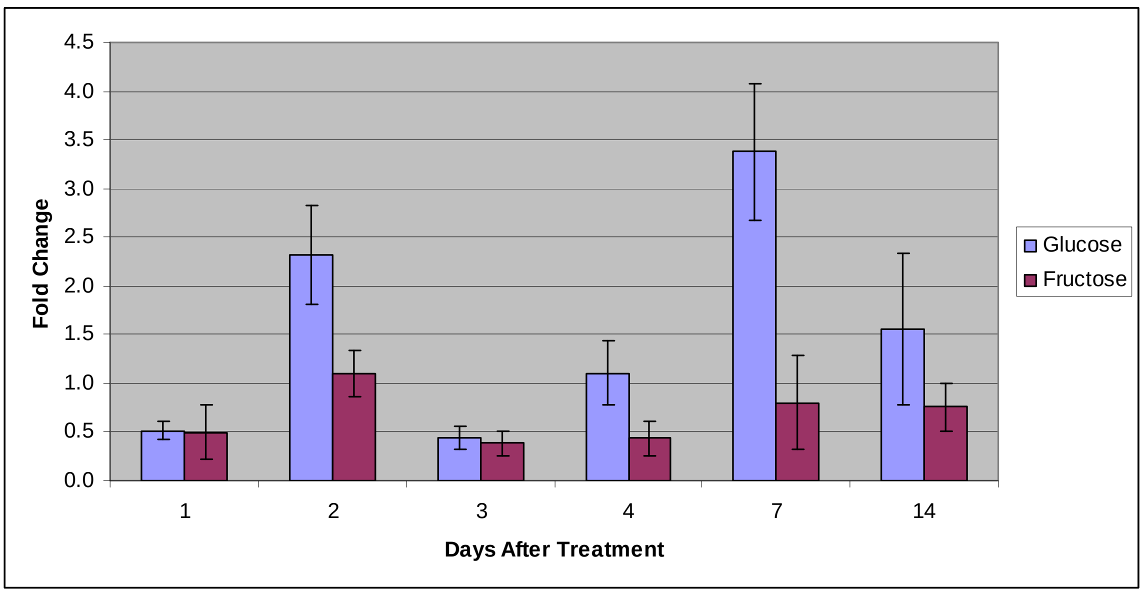


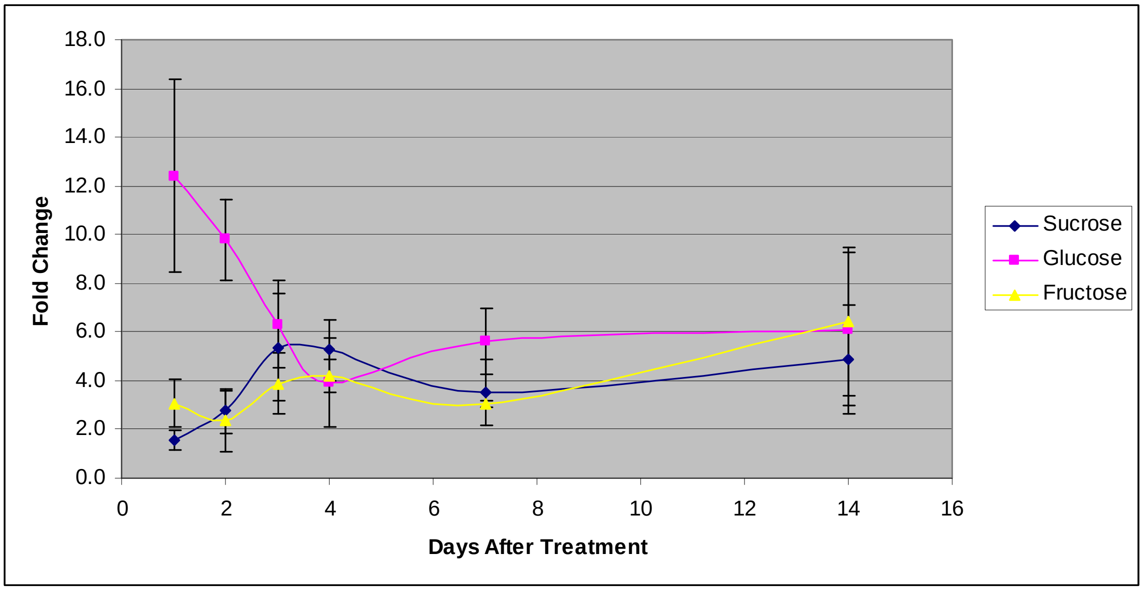
Figure 11. A, Levels of CYP71AV1 expression in sucrose, glucose and fructose treatments relative to pre-treatment at day 0. Day 0 level = 1.0; B, Levels of CYP71AV1 expression in glucose and fructose treatments relative to sucrose treatment. Sucrose level = 1.0.

Relative changes in transcript levels of the MVA and MEP early pathway genes were also measured. Expression levels of HMGR, DXR and DXS in sucrose-treated seedlings did not change significantly from those measured in pre-treated seedlings for all time points measured (Figures 14A-16A, respectively). FPS transcript levels, on the other hand, were significantly increased by day 2 in sucrose-fed seedlings when compared with those in pre-treated seedlings, and remained so for the duration of the experiment (Figure 12A).

HMGR, FPS, DXR, and DXS (Figures 13B-16B, respectively) all showed an initial increase in transcript levels in the glucose-treated seedlings compared to sucrose. With the exception of HMGR, transcript levels of the four early pathway genes remained significantly greater at day 2 in glucose compared to sucrose (Figures 14B-16B). On the other hand, at day 2 HMGR transcript levels in glucose-treated seedlings dropped to levels similar to sucrose (Figure 13B). After day 2, transcript levels for FPS, HMGR, and DXR in glucose-treated seedlings were not statistically different from those in sucrose for the duration of the experiment. DXS expression was about 2.5-fold greater in glucose than that in sucrose at day 3; subsequently, levels in glucose-treated seedlings dropped to those grown in sucrose in day 4 and remained statistically similar.

Fructose had no apparent effect on DXR or DXS. Transcript levels in fructose-treated seedlings were not statistically different from sucrose for the duration of the experiment (Figures 15B and 16B). Transcript levels of HMGR and FPS in fructose-treated seedlings, however, were significantly greater than those in sucrose-treated seedlings during day 1 (Figures 13B and 14B). FPS transcript levels decreased in fructose-treated seedlings to levels similar to sucrose at day 2, and were not statistically different for the remainder of the experiment. HMGR mRNA levels during day 2 in fructose, on the other hand, deceased compared to sucrose. After day 2, levels of HMGR transcripts in fructose-treated seedlings were not statistically different from sucrose.

A.



B.

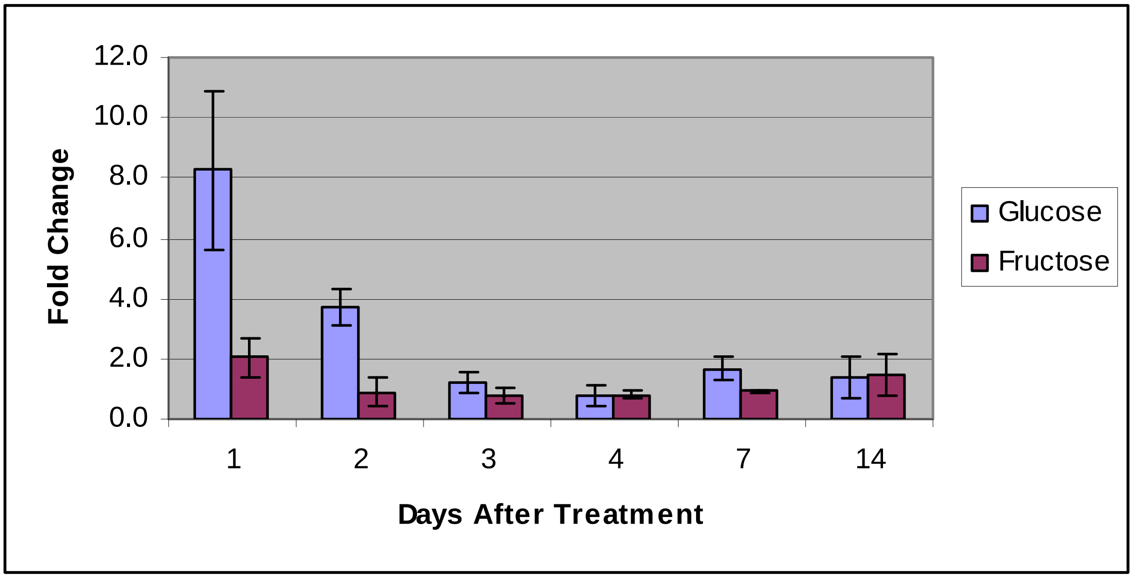
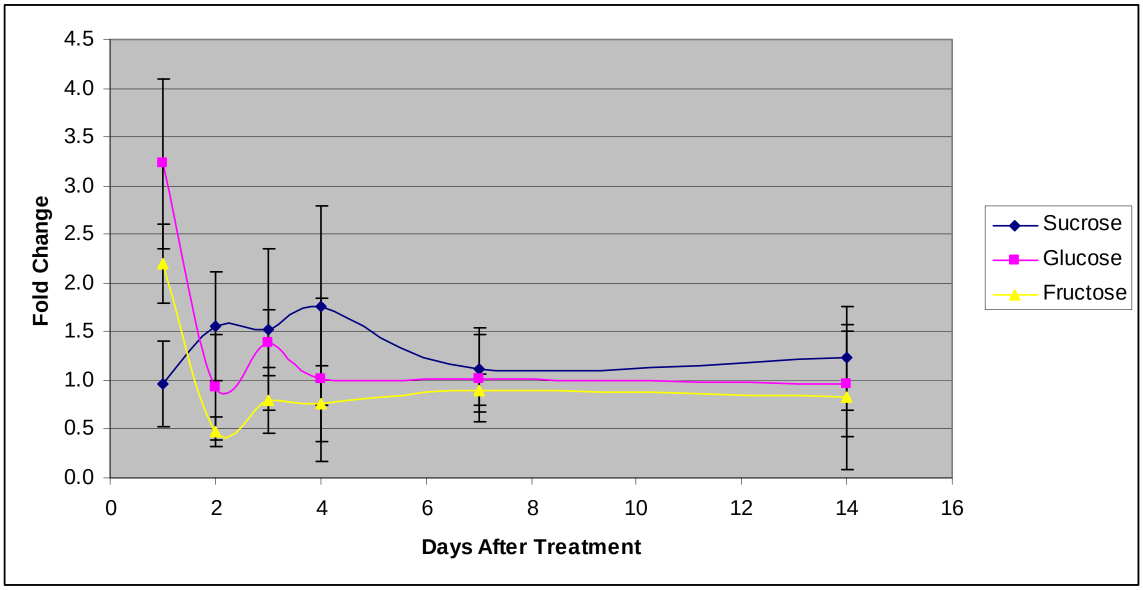


Figure 12. A, Levels of FPS expression in sucrose, glucose and fructose treatments relative to pre-treatment at day 0. Day 0 level = 1.0; B, Levels of FPS expression in glucose and fructose treatments relative to sucrose treatment. Sucrose level = 1.0.

A.



B.

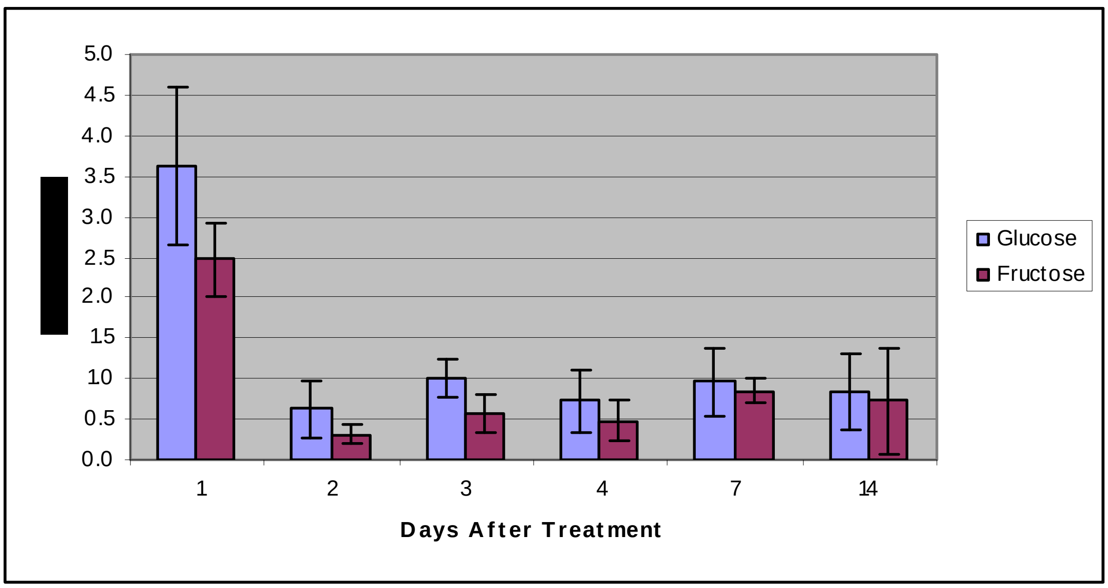
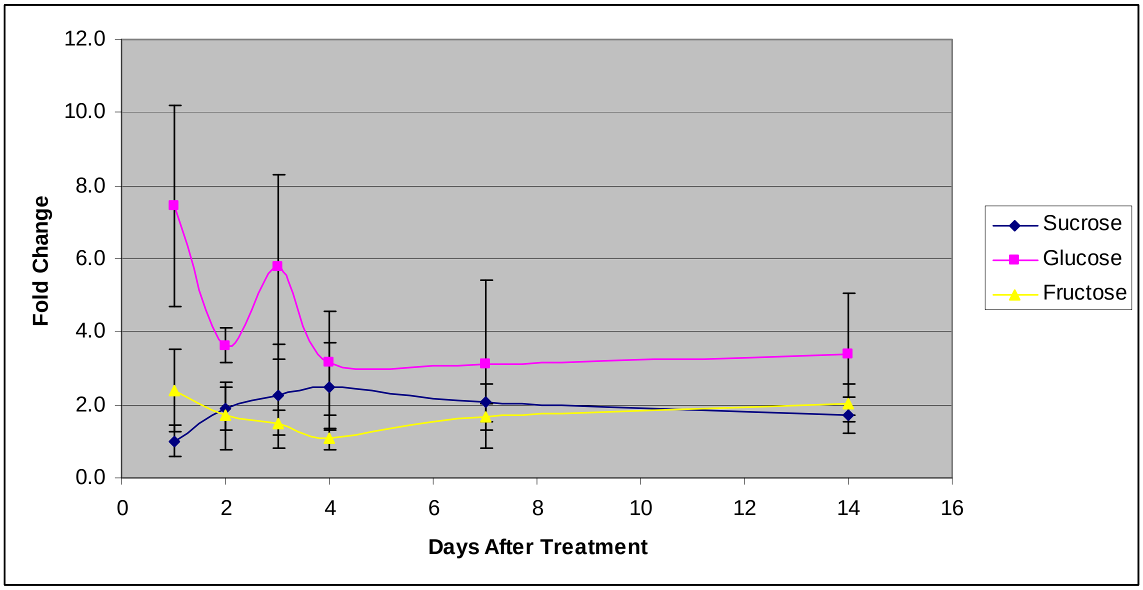


Figure 13. A, Levels of HMGR expression in sucrose, glucose and fructose treatments relative to pre-treatment at day 0. Day 0 level = 1.0; B, Levels of HMGR expression in glucose and fructose treatments relative to sucrose treatment. Sucrose level = 1.0.

A.



B.

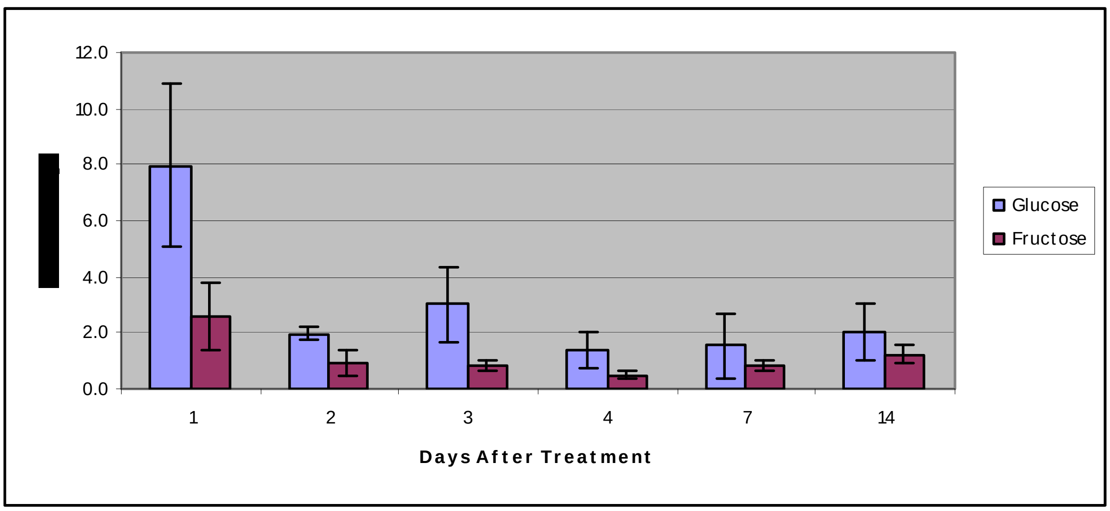
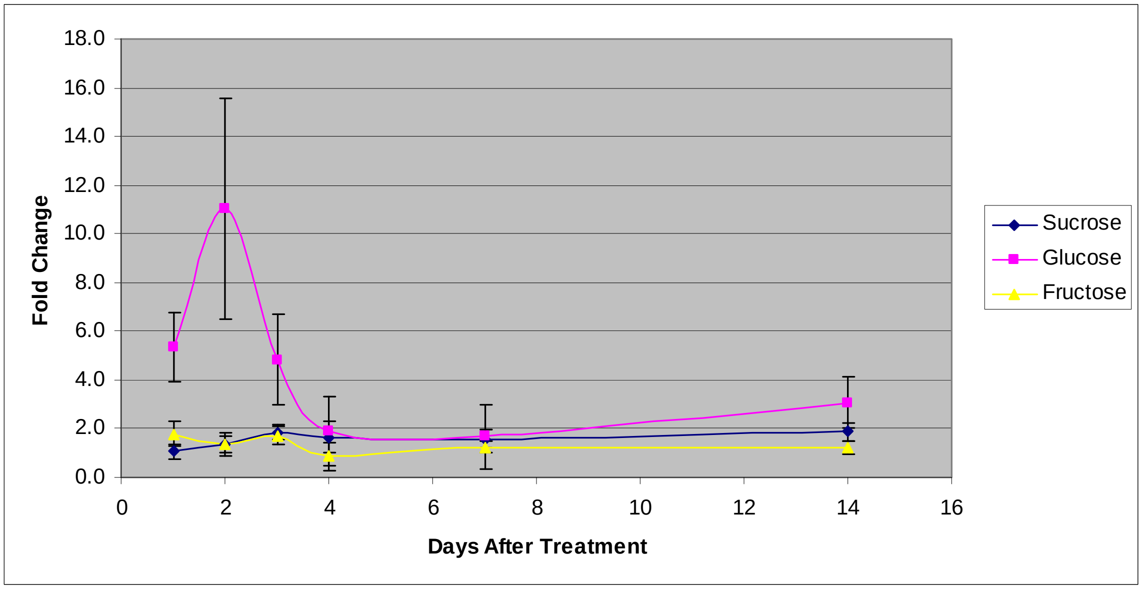


Figure 14. A, Levels of DXR expression in sucrose, glucose and fructose treatments relative to pre-treatment at day 0. Day 0 level = 1.0; B, Levels of DXR expression in glucose and fructose treatments relative to sucrose treatment. Sucrose level = 1.0.

A.



B.

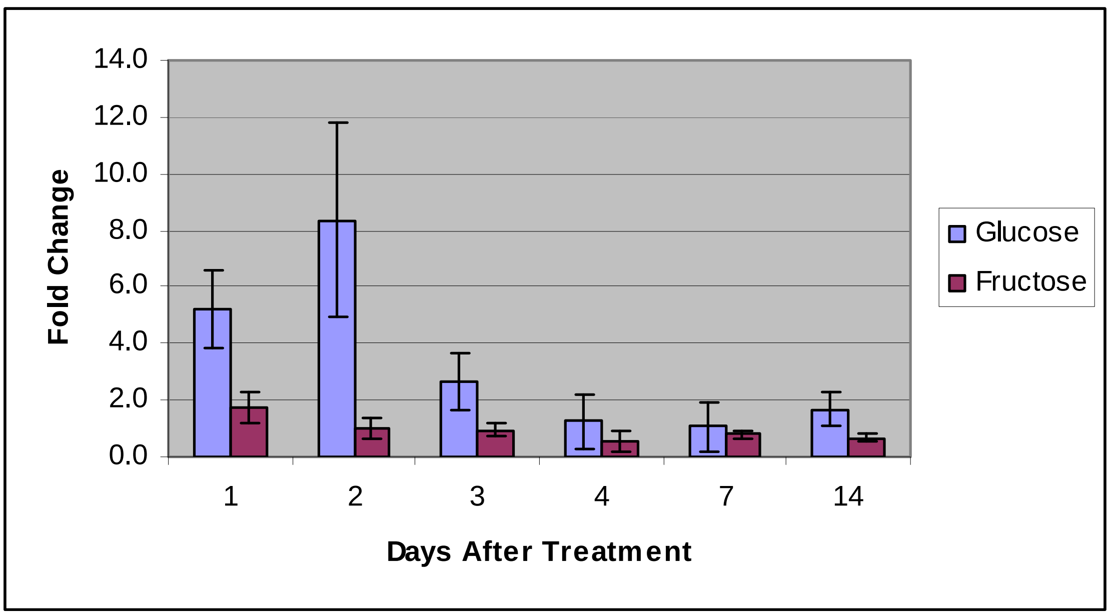


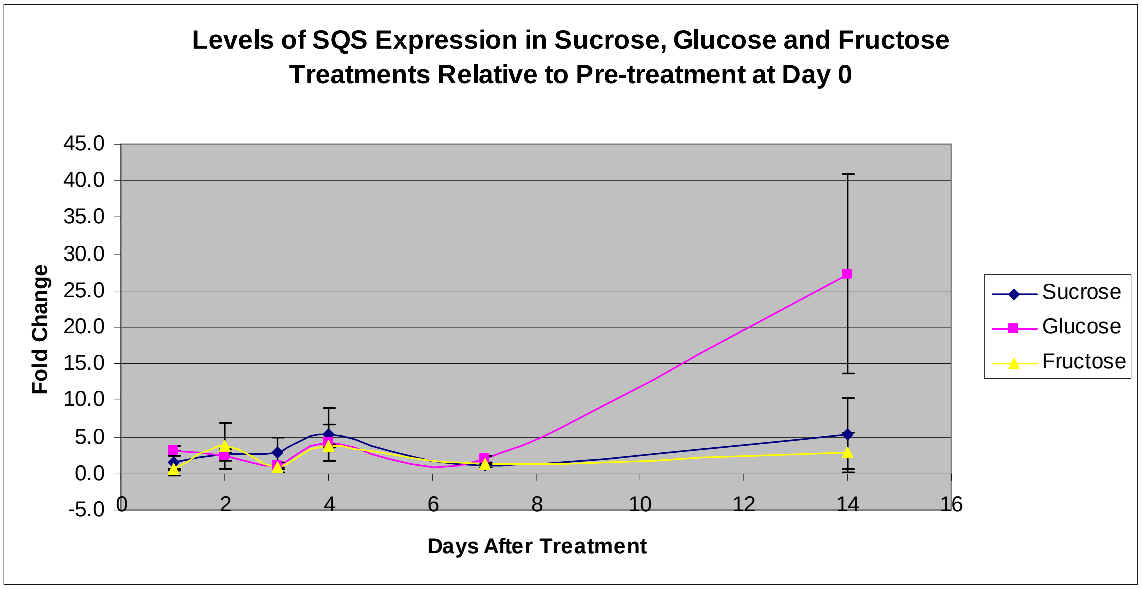
Figure 15. A, Levels of DXS expression in glucose and fructose treatments relative to pre-treatment at day 0. Day 0 level = 1.0; B, Levels of DXS expression in glucose and fructose treatments relative to sucrose treatment. Sucrose level = 1.0.

4.3. Sugars Do Not Appear to Initiate Coordinate Control Between Sterol and Sesquiterpene Pathways

Because many plants exhibit coordinate control between the sterol and sesquiterpene biosynthetic pathways, expression of squalene synthase (SQS) was also measured and compared to ADS in response to sugars. Levels of SQS transcripts in sucrose-fed seedlings were significantly increased by day 2 when compared with those in pre-treated seedlings (Figure 16A). Transcript levels in sucrose-fed seedlings seemed to remain significantly greater than those in pre-treated seedlings in day 3; however the results are not significant due to a large degree of variation. Levels in sucrose-fed seedlings remained significantly increased in day 4; however levels decreased during day 7 and were not significantly different from those in pre-treated seedlings. Transcript levels seemed to rise again in day 14 in sucrose-fed seedlings, but similar to day 3, there was a high degree of variation and the results are not significantly different from transcript levels in pre-treated seedlings.

When seedlings were grown in glucose, SQS increased almost 4 fold within 24 hours (Figure 16B). Transcripts decreased shortly thereafter and remained equivalent to those measured in seedlings grown in sucrose until day 14 when SQS mRNA increased 7 fold. There was no significant difference in mRNA transcripts between seedlings grown in fructose and those grown in sucrose. In contrast to our expectations, the steady increase in ADS transcripts was not accompanied by a steady decrease in SQS mRNA levels (Figure 17). Thus, it appears that glucose does not initiate the inverse coordinate control of the two opposing pathways similarly to that observed during exposure to fungal elicitors. These results suggest that glucose does not inversely regulate ADS and SQS in *A. annua*.

A.



B.

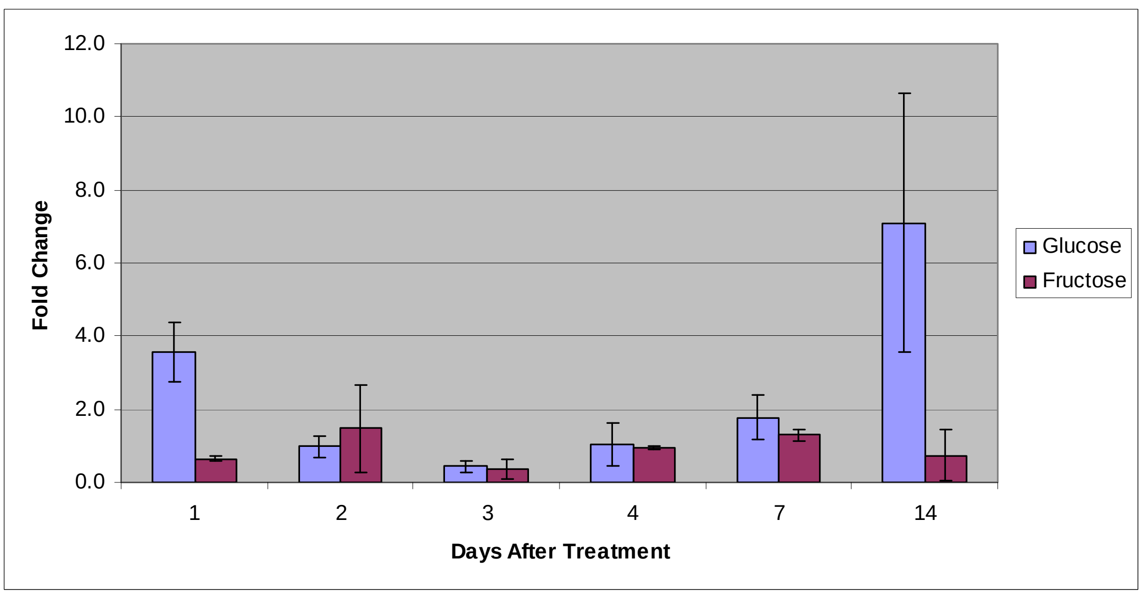


Figure 16. A, Levels of SQS expression in sucrose, glucose and fructose treatments relative to pre-treatment at day 0. Day 0 level = 1.0; B, Levels of SQS expression in glucose and fructose treatments relative to sucrose treatment. Sucrose level = 1.0.

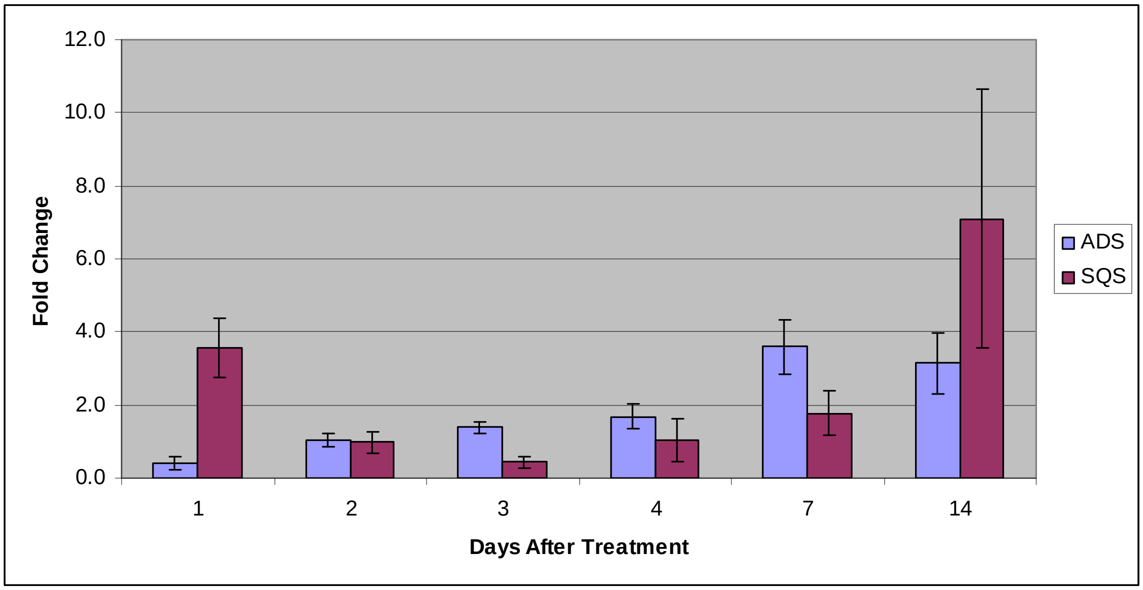


Figure 17. Levels of ADS and SQS expression in glucose treatment relative to sucrose treatment. Sucrose level = 1.0.

5. Discussion

5.1. Developmental Regulation of Artemisinin Biosynthesis

5.1.1. Early Cytosolic Pathway Genes are Highly Expressed in Reproductive Plants

HMGR is responsible for the formation of mevalonate and is the first committed step towards isoprenoids in all eukaryotes. Certain isoforms of this gene are known to accumulate in floral tissue and are probably important in flower development (Enjuto et al., 1995; Korth et al., 1997). This study has shown that HMGR expression is very highly upregulated in plants that shift into a reproductive growth stage (Figure 8). However, HMGR is known to be regulated post-transcriptionally, in particular by SnRK1 (Hey et al., 2006). Phosphorylation of HMGR by this kinase causes inactivation. Although levels of HMGR transcripts are remarkably increased, a consequent increase in enzyme activity and carbon flux may not necessarily accompany increased transcript levels. In transgenic plants over-expressing HMGR, very high levels of HMGR transcripts were not accompanied by an increase in enzyme activity (Re et al., 1995).

The suggestion of increased carbon flux through the early cytosolic pathway is further reinforced by results showing that the level of FPS transcripts also increases dramatically in budding plants (Figure 8). FPS produces the precursor for sesquiterpenoids, many of which are phytoalexins. During flowering, the plant is in a vital and vulnerable phase, therefore the need for defense compounds is much higher. In Arabidopsis, the FPS2 isoform is specific for certain tissues during certain stages of development, and it accumulates to its highest levels in floral tissue during early flower development (Cunillera et al., 2000). Levels of FPS2 subsequently decrease after pollination. Our results parallel the results of Cunillera et al. (2000), showing a dramatic increase in FPS transcripts levels during early flower development and budding with subsequent decrease during the period of full flowering.

5.1.2. Late Pathway Genes Are Upregulated During Flower Budding, But Not During Full Flower

The physiological role of artemisinin *in planta* is not clear; however it has been shown to have allelopathic properties, such as inhibiting seed germination, seedling growth and root induction in a variety of other plant species (Chen and Leather, 1989). Artemisinin has previously been shown to accumulate to high levels in seeds, therefore an upregulation of artemisinin-specific gene transcripts is reasonable during reproduction. ADS and CYP71AV1, the two genes that are responsible for the specific biosynthesis of artemisinin, are both upregulated to comparable levels in budding plants relative to vegetative plants. These levels subsequently decreased during full flowering to levels found in vegetative plants (Figure 9). It is possible that artemisinin is synthesized at heightened levels during floral differentiation, rather than during full flowering, and is sequestered until seed formation. The degree of upregulation of ADS and CYP71AV1 does not match that of HMGR and FPS. This is not surprising since carbon routing through the MVA pathway leads to many other products in addition to artemisinin.

5.1.3. Early Plastidic Pathway Genes Are Downregulated During Full Flower, But Not During Flower Budding

The plastidic pathway was predicted to play an important role in the shift from vegetative to reproductive growth. Isoprenoids produced in the plastid are important for floral pigmentation (carotenoids) and fragrances (monoterpenes). Further, Towler and Weathers (2007) showed that inhibition of the MEP pathway significantly reduced artemisinin production. However, results from these studies show that the mRNA levels of key isoprenoid biosynthetic genes in the plastid, DXR and DXS, are unchanged in flower budding plants compared to vegetative plants, and they are also downregulated during full flowering. Photosynthesis in this upper portion of the shoot may likely assume a lesser role as the plant shifts to a reproductive phase. It is also possible that cytosolic IPP may provide a crosstalk source of isoprene (IPP) biosynthesis in the plastid, since the cytosolic pool of IPP is likely very large due to the large increases in HMGR transcripts. Furthermore, this study has only measured regulation at the level of transcript accumulation. MEP pathway genes are known to be regulated post-transcriptionally (Sauret-Gueto et al., 2006); therefore it is possible that, although levels of transcripts are unchanged as the plant shifts to a reproductive, flowering stage, post-translational regulation is occurring.

5.2. Sugar Regulation of Artemisinin Biosynthesis

5.2.1. The Effects of Glucose and Fructose on Artemisinin Production Are Reflected at the Level of ADS mRNA Accumulation

Previous work showed that compared to sucrose, glucose induced the production of artemisinin in *A. annua*, while fructose inhibited its production. This study showed that this differential effect on artemisinin production correlates with an increase in ADS mRNA levels, whose protein product catalyzes the first committed step towards artemisinin (Figure 10A). Although transcript levels initially decreased after 24 hours of treatment in glucose-treated seedlings when compared with those of glucose-treated seedlings, levels quickly recovered and reached a peak at day 7**.** Compared to transcript levels in pre-treated seedlings at day 7, transcript levels of ADS in sucrose-treated seedlings were >40-fold greater (Figure 10B), illustrating the magnitude of effect that glucose exerts on this highly committed step towards artemisinin. Conversely, levels of ADS transcripts remained low in fructose-treated seedlings until day 14, when levels were no different than those of sucrose-treated seedlings. The reason for the initial decrease in ADS transcripts in glucose-treated seedlings is not clear, however it may be due to a stress response in the seedlings as they were physically removed from sugar-free B5 medium and placed in a sugar-containing environment.

5.2.2. CYP71AV1 Showed a Biphasic Response to Glucose

CYP71AV1 was also differentially regulated according to which of the three sugars was present, however the response was atypical. Instead of a steady increase over the course of the experiment, expression of CYP71AV1 appeared to have a biphasic response in glucose-treated seedlings (Figure 11A). This response was not reflected in the expression level of CYP in sucrose-treated seedlings when compared to that of pre-treated seedlings at day 0 (Figure 11B). This suggests that the observed response was specific to glucose, and not a general trend due to the presence of exogenous sugars. Levels of transcripts reached a minor peak at day 2, followed by a dramatic decrease during day 3. Levels again rose at day 4 and reached a major peak at day 7, after which they again decreased at day 14. It is possible that CYP71AV1 regulation is being heavily influenced by seedling development at the level of transcript accumulation. The first peak may correspond to a transient glucose-initiated upregulation of CYP71AV1 in the cotyledons of the seedlings, which only lasted for one day. In this study, germinated seedlings developed their first true leaves about 4 days after sugar treatment; therefore a glucose-specific upregulation may have occurred during development of the true leaves. Clearly there is sugar-specific crosstalk between multiple pathways in developing plants and although this study does not conclusively demonstrate such an interaction, the biphasic expression CYP71AV1 may be due to interactions between both sugar and developmental signals.

5.2.3. Early Pathway Genes Are Initially Upregulated in Glucose

Glucose had a clear effect on the early genes in the terpenoid biosynthetic pathway and this effect was not exclusive to genes localized to the cytosol or the plastid. Unlike ADS (Figure 10), transcript levels for HMGR, FPS, DXR, and DXS (Figures 13-16) were significantly upregulated during the first day in seedlings treated with glucose compared to sucrose. Heightened transcript levels in glucose compared to sucrose persisted through day 2 for FPS, DXR, and through day 3 for DXS, after which levels declined and were subsequently not significantly different from those in sucrose for all four genes. Fructose did not have as profound an effect; however, when compared to sucrose, treatment with fructose did cause increases in transcript levels for FPS and HMGR on day 1. Unlike what was observed for ADS, expression of the four early pathway genes was not downregulated by fructose with one exception; during day 2, HMGR levels in seedlings grown in fructose were less than those grown in sucrose.

The difference in timing of changes in expression for the early pathway genes compared to the late pathway genes towards artemisinin is interesting. The early pathway genes seem to show a rapid upregulation in response to glucose that occurs within 24 hours. Since these genes act upstream of many metabolic processes relating to carbon usage, their rapid upregulation due to possible glucose-mediated signaling is reasonable. Once the cell senses a large concentration of glucose, it upregulates the necessary pathways to manage and utilize the excess glucose (Rolland et al., 2006; Gibson, 2005). Furthermore, there seems to be no bias towards either the cytosolic MVA pathway or the plastidic MEP pathway; both pathways show approximately similar responses to glucose.

The lack of inverse regulatory effects on the expression of early pathway genes by glucose and fructose is also interesting. It is possible that glucose-mediated signaling does not cause linear increases in transcript accumulation of these early pathway genes in response to the concentration of glucose. There may be a certain threshold response whereby excess glucose is sensed and causes an increase in transcript accumulation. On the other hand, previous work by Wang and Weathers (2007) demonstrated a linear and proportionate increase in artemisinin yield as the ratio of glucose to fructose increased, suggesting such a threshold response does not exist. This also does not explain the increase in expression of HMGR and FPS in the first day in fructose-treated seedlings compared to sucrose. The degree of upregulation in fructose-treated seedlings is not, however, as dramatic as that seen in glucose-treated seedlings. HMGR is the exception, in that there does not seem to be a statistical difference in the levels of transcripts in the first day in glucose and fructose-treated seedlings compared to sucrose. However, HMGR is also different in that changes in expression in glucose for FPS, DXR, and DXS are initially all about 8-fold greater than that in sucrose, while HMGR expression is 3.5-fold higher in glucose compared to sucrose at day 1. HMGR may simply be less attenuated to glucose signaling than the other genes. Although there are very few studies relating to HMGR regulation and sugar, glucose was shown to have no effect on HMGR expression in the plant fungal pathogen, *Gibberella fujikuroi* (Woitek et al., 1997).

For HMGR, DXS and DXR, the baseline measurement of transcript levels in sucrose-treated seedlings during all time points measured did not differ significantly from those in the pre-treated seedlings at day 0 (Figures 14B-16B, respectively). This suggests that levels of sucrose mobilized from starch during the 1 day period during which the seedlings were incubated in the dark in sugar-free media were sufficient to maintain levels of transcripts similar to those when exogenous sucrose was supplied. This is further evidence that the observed increase in transcripts of these three genes during day 1 when supplied with glucose is a specific response to that sugar. The level of transcripts of FPS in sucrose-treated seedlings, on the other hand, was significantly increased at day 2 compared to that in pre-treated seedlings, and remained greater in the sucrose-fed seedlings than the pre-treated seedlings throughout the course of experiment (Figure 12B). Similar to ADS and CYP, this suggests that an excess of carbon from exogenously supplied sucrose increased carbon flux through the post-IPP pathway, whereas carbon flux through the early cytosolic and plastidic pathways was more likely provided by mobilized sucrose from starch during the dark, sugar-starved period. Carbon-labeling studies would help to verify this hypothesis.

5.2.4. Glucose May Be Acting As a Signal in Artemisinin Biosynthesis

Glucose seems to be acting as a signal to upregulate the biosynthetic pathway leading to artemisinin. The reason for this upregulation of this specific secondary metabolite may be a possible role of artemisinin as a sink for reactive oxygen species (ROS), specifically singlet oxygen (Wallaart et al., 2000). Artemisinin has 5 oxygens in its molecular structure (Figure 1), including 2 that comprise the therapeutically important endoperoxide bridge (Mercer et al., 2007). The last steps in its biosynthesis are most likely photooxygenic, involving the spontaneous reaction of dihydroartemisinic acid with singlet oxygen, a reaction identical to the photooxidation of polyunsaturated fatty acids

(Wallaart et al., 1999b). Experimental data from Kim et al. (2001) provided evidence for a possible relation between oxygen and artemisinin. They showed that highly aerated hairy root culture of *A. annua* produced significantly more artemisinin than poorly aerated cultures (Kim et al., 2001).

Reactive oxygen species (ROS) are cytotoxic to cells and singlet oxygen, in particular, may act as an apoptogenic signal in plant cells; therefore, it is important that accumulation of these molecules be minimized (op den Camp et al., 2003; Wagner et al., 2004). Soluble sugars and glucose in particular, through their metabolism, are sources of oxidative stress and plants have developed many evolutionary mechanisms to protect themselves from ROS (Mittler et al., 2004; Couee et al., 2006). Although there is no evidence that the physiological role of artemisinin is to act as an anti-oxidant associated with the accumulation or metabolism of glucose, the results presented in this and previous studies (Weathers et al., 2004; Wang and Weathers, 2007) showing a relationship between artemisinin and sugar suggest that artemisinin may indeed be an ROS sink. The difference in response to sucrose, glucose and fructose is probably related to specific sugar signaling pathways and the relative abundance of each constituent monosaccharide. The results in this study suggest that glucose is the specific signal that causes changes in gene expression of ADS and CYP71AV1. When seedlings are exposed to sucrose, the sugar is inverted yielding one equivalent molecule of glucose and one equivalent molecule of fructose; this is despite the ability of the cell to isomerize glucose to fructose and use fructose in glycolysis. However, when only glucose is supplied in the media at equal molar concentrations to that of the sucrose treatment, then there is a shift in the relative number of glucose molecules sensed by the cell, and therefore glucose regulation is magnified. Conversely, when only fructose is present in the media at equal molar concentrations, little glucose-mediated regulation occurs. Indeed studies by Wang and Weathers (2007) have shown an increase in the ratio of glucose to fructose is reflected by a corresponding increase in artemisinin yield.

The alternative is that the observed increase in artemisinin yields and levels of gene transcripts specific for artemisinin biosynthesis is the result of a large supply of exogenous carbon source being presented to the plant, and various carbon utilization pathways are upregulated. Artemisinin is a secondary metabolite, which by definition is not absolutely vital to the immediate survival of the plant and therefore is only synthesized when adequate amounts of carbon are available. However this is unlikely because the sucrose and the fructose media had the same amount of carbon as the glucose media, and would only be the case if fructose is not as efficiently used in metabolism as glucose.

It is also important to consider the effects of sugar depletion in the experimental media. Sugars play a dual role as both a signal and a metabolic substrate. Although the exogenous sugars are being sensed, they are also being metabolized or sequestered into starch granules and the concentrations of those sugars in the media is depleted over time. It is possible that by day 14, significant levels of sugars have been metabolized and their regulatory effects are thus diminished. The expression level of ADS is consistent with this logic. Fructose, on the other hand, caused ADS expression to recover to levels similar to that in sucrose-treated seedlings. This might suggest fructose is acting as a negative regulator of ADS, and only once the sugar begins to decrease in the medium does its inhibitory effects on ADS expression vanish. To our knowledge, however, no fructose-specific signaling pathways have been identified. What is probably occurring is that as the seedlings develop, fructose is likely depleted from the media while glucose is being generated through photosynthesis, and, thus, gradually re-establishing cellular responses due to glucose-specific signaling pathways.

5.3. SQS Does Not Appear to be Coordinately Controlled in Flower Development or in Response to Sugars

The post-FDP branch point represents an important decision in plants: should it invest carbon in growth or in the production of various secondary metabolites, many of which are important in defense. In some instances this decision needs to be made rapidly, and therefore the most efficient mechanism to direct carbon flow is upregulation of one pathway concomitant with downregulation of the other. This has been demonstrated to occur with SQS and an SQC relating to synthesis of phytoalexins (Vogeli and Chappell, 1988).

Since artemisinin has been shown to be upregulated in glucose treatment and when the plant shifts into reproductive growth, coordinate decreases in SQS transcript were anticipated. This did not, however, appear to be the case for either experimental condition. When compared to vegetative growth, SQS expression did not change in budding or flowering plants. Levels of SQS transcripts in glucose- and fructose-treated seedlings remained comparable to those in sucrose-treated seedlings for the duration of the sugar experiment with two exceptions: at day 3, SQS expression was downregulated in both glucose and fructose treatments, and at day 14, glucose caused an approximately 7 fold increase in SQS expression compared to sucrose. The increase in SQS transcripts at day 14, however, may be due to aforementioned sugar depletion effects. A 7 fold increase in SQS expression, thus, seems an unlikely effect of glucose depletion. Glucose signaling is also an unlikely cause of the observed increase in SQS expression. Any changes in gene expression directly due to a perception of signal are expected to occur long before 7 days.

SQS and SQC regulatory coordination has been observed in rapid defense responses, upon wounding and elicitation, however, those conditions were not tested in this study. As the plant shifts towards reproduction there is likely no perceived urgent need to produce sesquiterpenes at the expense of sterols. Indeed, there may be a need for more sterols as flowers develop and embryos are produced. If artemisinin is indeed acting as a quencher of singlet oxygen, then the danger posed by ROS might induce the cell to rapidly shift carbon balance from sterol synthesis to artemisinin synthesis if a large amount of glucose were suddenly available, however the results do not support this.

5.4. Conclusions

This study was the first to show that terpenoid genes relating to artemisinin biosynthesis are regulated at the level of transcript accumulation throughout the developmental shift from vegetative growth to flower budding and full flowering. The actual differentiation of floral tissues during flower budding seems to be the critical stage of biosynthesis of terpenoids necessary for the reproductive stage of the plant. This study demonstrated that during this stage the greatest levels of gene upregulation were observed. Furthermore, the early cytosolic pathway seems to be the major contributor of intermediates to terpenoid biosynthesis.

This study was also the first to demonstrate that specific terpene genes are being upregulated in their mRNA transcript accumulation, thus, providing further evidence that glucose is acting as a specific signal for artemisinin biosynthesis. Futhermore, this study showed for the first time that glucose-mediated signaling was not specific to either localized pathway, as upregulation of gene expression was observed in both cytosolic and plastidic pathways.

We also provided evidence that artemisinin in particular has an important role in both success of reproduction and management of excess glucose metabolism. In both the shift to reproduction and treatment with glucose, expression of artemisinin specific genes was upregulated. However, artemisinin is not synthesized at the expense of sterol synthesis, as this study showed that there was no apparent coordinate regulation of ADS and SQS gene expression.

5.5. Future Work

To further clarify and strengthen the results presented in this study, there are several avenues of future research. First, artemisinin yields must be measured for all time points and conditions in which relative levels of transcript accumulation were measured. Correlation of expression data and artemisinin levels is vital to establishing physiological relevance to the results presented in this study. Changes in relative levels of transcripts may not necessarily be reflected in production of artemisinin, as there are clearly many downstream steps that afford opportunities for regulation.

The roots clearly play a role in the biosynthesis of artemisinin. Although artemisinin is not synthesized or accumulated in root tissue, artemisinin-specific genes are expressed at low levels in root tissue (Teoh et al., 2006), and un-rooted shoot cultures of *A. annua* synthesize far less artemisinin when compared with rooted shoot cultures (Feirreira and Janick, 1996). It would be interesting to analyze terpene gene expression in the roots in parallel with the shoots to establish what, if any, role the roots have in artemisinin biosynthesis.

HMGR, DXS, and FPS are known to have multiple isoforms in plants (Choi et al., 1992; Kim et al., 2005; Cunillera et al., 2000). Although the number of isoforms is not known for *A. annua*, there are certainly more than one for these early pathway genes. The existence of isoform families in biosynthetic pathways allows for highly specific spatial, temporal, and conditional regulation. Identification, characterization, and expression profiling of the different isoforms of early terpenoid biosynthetic genes in *A. annua* would provide high resolution information on the specific regulation of transcript levels as the plant shifts from vegetative growth to flower budding and full flowering and in response to glucose and fructose compared to sucrose.

Finally, carbon-labeling studies would establish the precise metabolic flux of carbon through the pathway. Clearly, both early compartmentalized pathways can play a role in artemisinin biosynthesis. Correlation of radio-labeled substrate feeding results and relative expression levels would strengthen the results provided in this study and provide quantitative analysis of the relative importance of each pathway in artemisinin biosynthesis.

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APPENDIX

Table A1. Real-time PCR data and calculation of fold change and standard deviation for vegetative, budding and flowering plants.

Tissue	Gene	Dev. Stage	Sample	CT 1	CT 2	CT 3	Avg CT	18S CT 1	18S CT 2	18S CT3	Avg 18S CT	ΔCT	Avg Veg ΔCT	Fold Change	Avg Fold Change	Deviation	Deviation^2	ΣDeviation^2	SD
Shoots	ADS	Vegetative	1	23.9	23.9	23.8	**23.9**	12.6	12.4	12.6	**12.5**	11.3	11.8	1.4	**1.1**	0.3	0.1		
			2	29.2	29.1	29.1	**29.1**	16.4	16.2	16.4	**16.3**	12.8	11.8	0.5	**1.1**	-0.6	0.3		
			3	25.6	25.8	26.3	**25.9**	14.8	14.5	14.6	**14.6**	11.3	11.8	1.5	**1.1**	0.4	0.2		
			4	27.7	27.2	26.9	**27.3**	15.7	15.9	16.1	**15.9**	11.4	11.8	1.4	**1.1**	0.3	0.1		
			5	22.2	22.7	23.1	**22.7**	10.5	10.1	10.2	**10.3**	12.4	11.8	0.7	**1.1**	-0.4	0.2	0.8	**0.5**
		Budding	1	29.8	29.7	29.4	**29.6**	20.9	20.4	20.7	**20.7**	9.0	11.8	7.3	**6.3**	1.0	1.0		
			2	27.9	28.0	28.5	**28.1**	18.5	18.2	18.8	**18.5**	9.6	11.8	4.6	**6.3**	-1.7	2.9		
			3	29.6	30.3	29.5	**29.8**	20.3	20.2	20.0	**20.2**	9.6	11.8	4.6	**6.3**	-1.7	2.9		
			4	29.4	29.6	29.4	**29.5**	20.3	20.6	20.5	**20.5**	9.0	11.8	7.1	**6.3**	0.8	0.7		
			5	27.3	27.7	27.6	**27.5**	18.5	18.9	18.6	**18.7**	8.9	11.8	7.8	**6.3**	1.5	2.3	9.8	**1.6**
		Flowering	1	32.0	32.7	33.5	**32.7**	20.5	20.2	20.4	**20.4**	12.4	11.8	0.7	**0.6**	0.1	0.0		
			2	33.2	32.9	32.6	**32.9**	19.6	20.0	20.5	**20.0**	12.9	11.8	0.5	**0.6**	-0.1	0.0		
			3	31.9	31.1	31.7	**31.6**	19.4	18.9	19.3	**19.2**	12.4	11.8	0.7	**0.6**	0.1	0.0		
			4	29.7	30.3	29.7	**29.9**	17.1	16.8	17.2	**17.0**	12.9	11.8	0.5	**0.6**	-0.1	0.0	0.0	**0.1**
	CYP	Vegetative	1	24.6	24.5	24.8	**24.6**	12.6	12.4	12.6	**12.5**	12.1	12.7	1.5	**1.1**	0.4	0.2		
			2	30.2	30.1	30.1	**30.1**	16.4	16.2	16.4	**16.3**	13.8	12.7	0.5	**1.1**	-0.7	0.4		
			3	27.0	26.5	26.6	**26.7**	14.8	14.5	14.6	**14.6**	12.1	12.7	1.6	**1.1**	0.4	0.2		
			4	27.9	27.9	28.5	**28.1**	15.7	15.9	16.1	**15.9**	12.2	12.7	1.4	**1.1**	0.3	0.1		
			5	23.6	23.6	23.8	**23.7**	10.5	10.1	10.2	**10.3**	13.4	12.7	0.6	**1.1**	-0.5	0.3	1.1	**0.5**
		Budding	1	30.8	31.4	30.5	**30.9**	20.9	20.4	20.7	**20.7**	10.2	12.7	5.6	**6.6**	-1.1	1.1		
			2	28.9	29.0	28.9	**28.9**	18.5	18.2	18.8	**18.5**	10.4	12.7	4.9	**6.6**	-1.8	3.2		
			3	30.7	31.0	30.6	**30.8**	20.3	20.2	20.0	**20.2**	10.6	12.7	4.3	**6.6**	-2.3	5.4		
			4	30.1	30.3	30.1	**30.2**	20.3	20.6	20.5	**20.5**	9.7	12.7	8.1	**6.6**	1.4	2.0		
			5	28.4	27.7	27.9	**28.0**	18.5	18.9	18.6	**18.7**	9.3	12.7	10.4	**6.6**	3.8	14.1	25.9	**2.5**
		Flowering	1	33.1	33.2	33.0	**33.1**	20.5	20.2	20.4	**20.4**	12.7	12.7	1.0	**1.8**	-0.8	0.7		
			2	32.6	32.5	32.3	**32.5**	19.6	20.0	20.5	**20.0**	12.4	12.7	1.2	**1.8**	-0.6	0.4		
			3	30.5	30.4	30.6	**30.5**	19.4	18.9	19.3	**19.2**	11.3	12.7	2.7	**1.8**	0.8	0.7		
			4	28.1	28.7	28.6	**28.5**	17.1	16.8	17.2	**17.0**	11.4	12.7	2.4	**1.8**	0.6	0.4	2.1	**0.8**
	FPS	Vegetative	1	24.0	24.1	24.0	**24.0**	10.8	10.7	11.0	**10.8**	13.2	13.3	1.1	**1.1**	0.0	0.0		
			2	28.2	28.0	27.8	**28.0**	14.7	14.6	14.3	**14.5**	13.5	13.3	0.9	**1.1**	-0.2	0.0		
			3	25.6	26.0	25.8	**25.8**	12.9	12.9	13.2	**13.0**	12.8	13.3	1.5	**1.1**	0.3	0.1		
			4	26.8	27.2	27.5	**27.2**	14.6	14.4	14.8	**14.6**	12.6	13.3	1.7	**1.1**	0.6	0.4		
			5	24.9	24.5	24.4	**24.6**	9.8	10.0	10.0	**9.9**	14.7	13.3	0.4	**1.1**	-0.7	0.5	1.0	**0.5**
		Budding	1	30.6	30.0	30.3	**30.3**	25.7	25.5	25.6	**25.6**	4.7	13.3	398.9	**404.2**	-5.3	28.0		
			2	28.5	28.2	28.6	**28.4**	23.4	23.2	23.4	**23.3**	5.1	13.3	302.3	**404.2**	-101.9	10382.3		
			3	30.3	29.6	30.0	**30.0**	24.9	24.7	25.0	**24.9**	5.1	13.3	302.3	**404.2**	-101.9	10382.3		
			4	30.4	29.8	29.7	**30.0**	25.6	25.6	25.8	**25.7**	4.3	13.3	526.4	**404.2**	122.2	14924.7		
			5	28.7	28.9	29.1	**28.9**	24.5	24.6	24.4	**24.5**	4.4	13.3	491.1	**404.2**	86.9	7554.3	43271.6	**104.0**
		Flowering	1	31.7	31.1	31.4	**31.4**	24.4	24.7	24.4	**24.5**	6.9	13.3	86.8	**104.5**	-17.7	312.4		
			2	30.7	30.7	30.7	**30.7**	24.0	24.4	24.5	**24.3**	6.4	13.3	122.8	**104.5**	18.3	334.5		
			3	29.8	30.0	30.3	**30.0**	22.9	22.8	22.6	**22.8**	7.3	13.3	67.3	**104.5**	-37.2	1380.9		
			4	28.2	28.5	27.8	**28.2**	22.1	21.8	22.0	**22.0**	6.2	13.3	141.0	**104.5**	36.5	1335.6	3363.4	**33.5**
	HMGR	Vegetative	1	28.0	28.1	28.0	**28.0**	11.9	11.9	12.6	**12.1**	15.9	16.1	1.2	**1.4**	-0.3	0.1		
			2	32.3	32.6	32.0	**32.3**	15.8	15.9	15.6	**15.8**	16.5	16.1	0.8	**1.4**	-0.7	0.5		
			3	29.3	29.2	29.2	**29.2**	15.5	15.6	13.7	**14.9**	14.3	16.1	3.5	**1.4**	2.1	4.4		
			4	29.2	29.0	29.4	**29.2**	13.7	13.6	13.9	**13.7**	15.5	16.1	1.6	**1.4**	0.1	0.0		
			5	28.0	28.1	28.1	**28.1**	9.6	9.6	9.8	**9.7**	18.4	16.1	0.2	**1.4**	-1.2	1.5	6.5	**1.8**
		Budding	1	32.0	32.4	31.9	**32.1**	25.7	25.5	25.6	**25.6**	6.5	16.1	786.9	**1028.8**	-241.9	58527.7		
			2	29.6	30.0	30.2	**29.9**	23.4	23.2	23.4	**23.3**	6.6	16.1	734.2	**1028.8**	-294.6	86800.9		
			3	31.8	29.9	31.7	**31.1**	24.9	24.7	25.0	**24.9**	6.3	16.1	925.0	**1028.8**	-103.8	10772.3		

		4	30.7	31.2	33.2	**31.7**	25.6	25.6	25.8	**25.7**	6.0	16.1	1087.4	**1028.8**	58.6	3433.3		
		5	29.1	30.0	30.8	**30.0**	24.5	24.6	24.4	**24.5**	5.5	16.1	1610.5	**1028.8**	581.7	338421.5	497955.7	**352.8**
	Flowering	1	33.0	33.1	32.8	**33.0**	24.0	24.4	24.5	**24.3**	8.7	16.1	175.3	**130.1**	45.2	2039.7		
		2	33.0	33.3	33.3	**33.2**	22.9	22.8	22.6	**22.8**	10.4	16.1	51.5	**130.1**	-78.6	6176.2		
		3	30.6	30.8	30.8	**30.7**	22.1	21.8	22.0	**22.0**	8.8	16.1	163.5	**130.1**	33.4	1117.3	9333.2	**68.3**
DXR	Vegetative	1	26.2	26.6	26.3	**26.4**	13.7	13.6	13.5	**13.6**	12.8	14.0	2.4	**1.1**	1.2	1.5		
		2	28.6	28.6	28.4	**28.5**	14.3	14.5	14.4	**14.4**	14.1	14.0	0.9	**1.1**	-0.2	0.0		
		3	26.9	27.1	27.5	**27.2**	12.8	12.7	12.8	**12.8**	14.4	14.0	0.8	**1.1**	-0.4	0.1		
		4	28.1	28.0	28.3	**28.1**	14.0	13.9	14.1	**14.0**	14.1	14.0	0.9	**1.1**	-0.2	0.0		
		5	24.2	24.3	24.1	**24.2**	9.7	9.6	9.6	**9.6**	14.6	14.0	0.7	**1.1**	-0.4	0.2	1.9	**0.7**
	Budding	1	23.1	23.3	23.7	**23.4**	9.5	9.5	9.5	**9.5**	13.9	14.0	1.1	**0.8**	0.3	0.1		
		2	23.5	23.7	23.8	**23.7**	9.4	9.3	9.2	**9.3**	14.4	14.0	0.8	**0.8**	0.0	0.0		
		3	23.8	23.6	24.2	**23.9**	9.1	9.1	9.2	**9.1**	14.7	14.0	0.6	**0.8**	-0.2	0.0		
		4	23.6	23.5	23.6	**23.6**	9.1	9.3	9.2	**9.2**	14.4	14.0	0.8	**0.8**	0.0	0.0		
		5	23.2	23.4	23.6	**23.4**	9.0	8.8	8.9	**8.9**	14.5	14.0	0.7	**0.8**	-0.1	0.0	0.1	**0.2**
	Flowering	1	25.3	25.2	25.7	**25.4**	9.9	9.7	9.8	**9.8**	15.6	14.0	0.3	**0.4**	-0.1	0.0		
		2	25.8	25.8	25.4	**25.7**	9.3	9.3	9.2	**9.3**	16.4	14.0	0.2	**0.4**	-0.3	0.1		
		3	24.3	24.3	24.7	**24.4**	9.3	9.3	9.6	**9.4**	15.0	14.0	0.5	**0.4**	0.0	0.0		
		4	23.9	24.0	24.1	**24.0**	9.4	9.3	9.3	**9.3**	14.7	14.0	0.6	**0.4**	0.2	0.0		
		5	23.6	23.7	23.9	**23.7**	8.9	9.0	8.8	**8.9**	14.8	14.0	0.6	**0.4**	0.1	0.0	0.1	**0.2**
DXS	Vegetative	1	26.9	26.9	27.1	**27.0**	13.7	13.6	13.5	**13.6**	13.4	14.6	2.4	**1.2**	1.2	1.5		
		2	29.1	29.2	29.8	**29.4**	14.3	14.5	14.4	**14.4**	15.0	14.6	0.8	**1.2**	-0.4	0.2		
		3	27.6	27.4	27.9	**27.6**	12.8	12.7	12.8	**12.8**	14.9	14.6	0.9	**1.2**	-0.4	0.1		
		4	27.9	28.0	28.0	**28.0**	14.0	13.9	14.1	**14.0**	14.0	14.6	1.6	**1.2**	0.4	0.1		
		5	25.5	25.5	26.0	**25.7**	9.7	9.6	9.6	**9.6**	16.0	14.6	0.4	**1.2**	-0.8	0.7	2.6	**0.8**
	Budding	1	24.8	25.1	25.1	**25.0**	10.1	10.0	10.1	**10.1**	14.9	14.6	0.8	**0.6**	0.2	0.1		
		2	24.1	24.3	24.6	**24.3**	9.3	9.1	9.2	**9.2**	15.1	14.6	0.7	**0.6**	0.1	0.0		
		3	24.9	24.7	24.7	**24.8**	8.8	8.8	8.9	**8.8**	15.9	14.6	0.4	**0.6**	-0.2	0.0		
		4	29.2	28.8	28.2	**28.7**	12.7	12.8	12.8	**12.8**	16.0	14.6	0.4	**0.6**	-0.2	0.0	0.1	**0.2**
	Flowering	1	26.6	26.8	27.3	**26.9**	10.0	10.3	10.2	**10.2**	16.7	14.6	0.2	**0.2**	0.0	0.0		
		2	27.2	27.1	26.6	**27.0**	9.2	9.3	9.0	**9.2**	17.8	14.6	0.1	**0.2**	-0.1	0.0		
		3	25.5	25.3	25.4	**25.4**	8.7	8.8	9.0	**8.8**	16.6	14.6	0.3	**0.2**	0.1	0.0		
		4	26.7	26.7	26.7	**26.7**	9.1	9.2	9.3	**9.2**	17.5	14.6	0.1	**0.2**	-0.1	0.0		
		5	25.3	25.6	25.9	**25.6**	8.6	8.7	8.6	**8.6**	17.0	14.6	0.2	**0.2**	0.0	0.0	0.0	**0.1**
SQS	Vegetative	1	25.0	25.1	24.7	**24.9**	10.8	10.7	11.0	**10.8**	14.1	13.9	0.9	**1.1**	-0.2	0.0		
		2	27.6	27.5	27.6	**27.6**	14.7	14.6	14.3	**14.5**	13.0	13.9	1.8	**1.1**	0.8	0.6		
		3	27.1	27.4	27.1	**27.2**	12.9	12.9	13.2	**13.0**	14.2	13.9	0.8	**1.1**	-0.3	0.1		
		4	28.1	28.0	28.6	**28.2**	14.6	14.4	14.8	**14.6**	13.6	13.9	1.2	**1.1**	0.1	0.0		
		5	24.2	24.5	24.8	**24.5**	9.8	10.0	10.0	**9.9**	14.6	13.9	0.6	**1.1**	-0.4	0.2	0.9	**0.5**
	Budding	1	22.8	22.6	23.2	**22.9**	9.5	9.5	9.5	**9.5**	13.4	13.9	1.5	**1.3**	0.1	0.0		
		2	22.7	22.5	22.4	**22.5**	9.4	9.3	9.2	**9.3**	13.2	13.9	1.6	**1.3**	0.3	0.1		
		3	22.4	22.7	22.9	**22.7**	9.1	9.1	9.2	**9.1**	13.5	13.9	1.3	**1.3**	0.0	0.0		
		4	22.6	22.5	23.9	**23.0**	9.1	9.3	9.2	**9.2**	13.8	13.9	1.1	**1.3**	-0.3	0.1		
		5	22.6	22.1	22.6	**22.4**	9.0	8.8	8.9	**8.9**	13.5	13.9	1.3	**1.3**	0.0	0.0	0.2	**0.2**
	Flowering	1	24.1	24.1	24.2	**24.1**	9.9	9.7	9.8	**9.8**	14.3	13.9	0.7	**0.6**	0.1	0.0		
		2	24.1	23.8	23.8	**23.9**	9.3	9.3	9.2	**9.3**	14.6	13.9	0.6	**0.6**	0.0	0.0		
		3	23.3	23.0	24.7	**23.7**	9.3	9.3	9.6	**9.4**	14.3	13.9	0.8	**0.6**	0.2	0.0		
		4	24.2	24.4	25.3	**24.6**	9.4	9.3	9.3	**9.3**	15.3	13.9	0.4	**0.6**	-0.2	0.1		
		5	23.2	23.4	23.9	**23.5**	8.9	9.0	8.8	**8.9**	14.6	13.9	0.6	**0.6**	0.0	0.0	0.1	**0.2**

Table A2. Real-time PCR data and calculation of fold change and standard deviation for glucose and fructose treatments relative to sucrose treatment at days 1, 2, 3, 4, 7, and 14.

Gene	Day	Treatment	Sample	CT 1	CT 2	CT 3	Avg CT	18S CT 1	18S CT 2	18S CT3	Avg 18S CT	ΔCT	Avg suc ΔCT	Fold Change	Avg Fold Change	Deviation	Deviation^2	ΣDeviation^2	SD
ADS	1	suc	1	33.2	32.1	33.2	32.8	19.3	19.1	19.1	19.2	13.7	13.4	0.8	1.0	-0.2	0.0		
			2	33.1	33.5	33.2	33.3	19.7	19.5	19.6	19.6	13.7	13.4	0.8	1.0	-0.2	0.0		
			3	30.2	30.4	31.1	30.6	17.6	17.6	17.7	17.6	12.9	13.4	1.4	1.0	0.4	0.1	0.2	0.3
		glc	1	33.2	32.7	32.2	32.7	17.0	17.1	17.0	17.0	15.7	13.4	0.2	0.4	-0.2	0.0		
			2	33.4	33.5	33.1	33.3	18.5	18.8	18.7	18.7	14.7	13.4	0.4	0.4	0.0	0.0		
			3	33.5	33.1	32.9	33.2	18.8	19.0	18.9	18.9	14.3	13.4	0.6	0.4	0.2	0.0	0.1	0.2
		fru	1	30.6	31.0	30.9	30.8	16.6	16.6	16.7	16.6	14.2	13.4	0.6	0.5	0.0	0.0		
			2	33.1	33.0	32.9	33.0	18.2	18.4	18.6	18.4	14.6	13.4	0.4	0.5	-0.1	0.0		
			3	29.8	29.9	30.2	30.0	16.0	15.9	15.4	15.8	14.2	13.4	0.6	0.5	0.0	0.0	0.0	0.1
	2	suc	1	29.7	29.6	29.9	29.7	18.3	18.4	18.7	18.5	11.3	11.0	0.8	1.0	-0.2	0.0		
			2	29.2	29.6	29.7	29.5	18.8	18.7	18.7	18.7	10.8	11.0	1.2	1.0	0.2	0.0		
			3	31.1	29.9	30.7	30.6	19.7	19.4	19.5	19.5	11.0	11.0	1.0	1.0	0.0	0.0	0.1	0.2
		glc	1	28.1	28.2	28.4	28.2	17.1	17.2	17.0	17.1	11.1	11.0	0.9	1.0	-0.1	0.0		
			2	26.9	26.9	26.9	26.9	16.4	16.0	16.2	16.2	10.7	11.0	1.3	1.0	0.2	0.0		
			3	29.8	30.0	29.9	29.9	18.7	18.8	18.8	18.8	11.1	11.0	0.9	1.0	-0.1	0.0	0.1	0.2
		fru	1	28.7	29.3	29.3	29.1	16.7	16.7	16.7	16.7	12.4	11.0	0.4	0.3	0.1	0.0		
			2	29.6	29.9	29.7	29.7	17.1	16.9	17.1	17.0	12.7	11.0	0.3	0.3	0.0	0.0		
			3	29.0	29.3	29.3	29.2	16.0	16.0	16.5	16.2	13.0	11.0	0.2	0.3	-0.1	0.0	0.0	0.1
	3	suc	1	33.4	33.3	33.4	33.4	22.7	23.0	23.1	22.9	10.4	10.5	1.1	1.0	0.1	0.0		
			2	24.3	24.3	24.4	24.3	13.8	13.6	13.7	13.7	10.6	10.5	0.9	1.0	-0.1	0.0		
			3	26.2	26.0	26.1	26.1	15.7	15.5	15.6	15.6	10.5	10.5	1.0	1.0	0.0	0.0	0.0	0.1
		glc	1	31.5	30.9	31.2	31.2	21.0	21.1	21.5	21.2	10.0	10.5	1.4	1.4	0.1	0.0		
			2	34.9	34.5	34.6	34.7	24.8	24.8	24.6	24.7	9.9	10.5	1.5	1.4	0.1	0.0		
			3	33.5	33.5	33.4	33.5	23.3	23.2	23.1	23.2	10.3	10.5	1.2	1.4	-0.2	0.0	0.1	0.2
		fru	1	33.3	33.3	33.3	33.3	20.7	20.8	20.9	20.8	12.5	10.5	0.3	0.3	0.0	0.0		
			2	34.3	34.5	34.4	34.4	21.6	21.8	21.5	21.6	12.8	10.5	0.2	0.3	0.0	0.0		
			3	34.5	34.0	34.1	34.2	21.8	22.0	21.9	21.9	12.3	10.5	0.3	0.3	0.0	0.0	0.0	0.0
	4	suc	1	31.6	31.6	31.6	31.6	21.5	21.5	21.4	21.5	10.1	10.0	0.9	1.0	-0.1	0.0		
			2	30.9	30.8	30.8	30.8	20.6	20.8	20.6	20.7	10.2	10.0	0.9	1.0	-0.1	0.0		
			3	24.9	24.8	24.8	24.8	14.8	15.1	15.3	15.1	9.8	10.0	1.2	1.0	0.2	0.0	0.1	0.2
		glc	1	26.4	26.4	26.4	26.4	16.7	16.8	16.8	16.8	9.6	10.0	1.3	1.7	-0.4	0.1		
			2	27.5	27.5	27.5	27.5	18.3	18.6	18.5	18.5	9.0	10.0	2.0	1.7	0.3	0.1		
			3	28.6	28.4	28.4	28.5	19.0	19.4	19.3	19.2	9.2	10.0	1.7	1.7	0.1	0.0	0.2	0.3
		fru	1	30.4	30.4	30.3	30.4	17.1	17.4	17.6	17.4	13.0	10.0	0.1	0.2	-0.1	0.0		
			2	31.1	31.1	31.1	31.1	19.0	18.8	18.7	18.8	12.3	10.0	0.2	0.2	0.0	0.0		
			3	33.4	33.4	33.3	33.4	20.9	21.2	21.3	21.1	12.2	10.0	0.2	0.2	0.0	0.0	0.0	0.1
	7	suc	1	26.0	26.7	25.8	26.2	15.5	15.8	16.1	15.8	10.4	10.3	0.9	1.0	-0.1	0.0		
			2	24.0	24.5	24.7	24.4	14.5	14.5	14.6	14.5	9.9	10.3	1.3	1.0	0.3	0.1		
			3	28.0	28.0	27.9	28.0	17.7	16.7	17.9	17.4	10.5	10.3	0.8	1.0	-0.2	0.0	0.1	0.3
		glc	1	23.7	23.7	23.8	23.7	15.0	15.8	15.9	15.6	8.2	10.3	4.3	3.6	0.7	0.4		
			2	24.0	24.2	24.3	24.2	15.9	15.1	15.2	15.4	8.8	10.3	2.8	3.6	-0.8	0.6		
			3	26.0	25.9	25.9	25.9	17.7	17.3	17.7	17.6	8.4	10.3	3.7	3.6	0.1	0.0	1.1	0.7
		fru	1	26.5	26.4	26.6	26.5	15.6	15.2	14.8	15.2	11.3	10.3	0.5	0.5	0.0	0.0		
			2	26.1	26.0	25.9	26.0	14.7	15.4	15.6	15.2	10.8	10.3	0.7	0.5	0.2	0.0		
			3	26.8	26.9	26.9	26.9	15.5	15.6	14.8	15.3	11.6	10.3	0.4	0.5	-0.1	0.0	0.0	0.2
	14	suc	1	23.9	23.9	24.5	24.1	14.3	14.2	14.1	14.2	9.9	9.9	1.0	1.0	0.0	0.0		
			2	25.2	24.9	25.6	25.2	14.7	15.5	14.8	15.0	10.2	9.9	0.8	1.0	-0.2	0.1		
			3	24.5	24.6	24.9	24.7	14.9	15.0	15.7	15.2	9.5	9.9	1.3	1.0	0.3	0.1	0.2	0.3
		glc	1	23.2	22.8	23.1	23.0	14.7	14.6	14.7	14.7	8.4	9.9	2.8	3.1	-0.3	0.1		
			2	21.6	21.6	21.6	21.6	13.8	13.9	13.6	13.8	7.8	9.9	4.1	3.1	0.9	0.9		

			3	22.3	22.4	22.3	**22.3**	13.7	13.8	13.9	**13.8**	8.5	9.9	2.5	**3.1**	-0.6	0.4	1.4	**0.8**
		fru	1	24.2	24.6	24.9	**24.6**	14.8	14.8	15.1	**14.9**	9.7	9.9	1.1	**0.8**	0.4	0.1		
			2	25.1	25.0	24.8	**25.0**	14.7	14.5	14.4	**14.5**	10.4	9.9	0.7	**0.8**	-0.1	0.0		
			3	25.3	25.5	25.5	**25.4**	14.6	14.7	14.6	**14.6**	10.8	9.9	0.5	**0.8**	-0.3	0.1	0.2	**0.3**

Gene	Day	Treatment	Sample	CT 1	CT 2	CT 3	Avg CT	18S CT 1	18S CT 2	18S CT3	Avg 18S CT	ΔCT	Avg suc ΔCT	Fold Change	Avg Fold Change	Deviation	$Deviation^2$	$\Sigma Deviation^2$	SD
CYP	1	suc	1	32.3	33.1	32.7	**32.7**	19.3	19.1	19.4	**19.3**	13.4	13.3	0.9	**1.0**	-0.1	0.0		
			2	33.3	33.4	33.7	**33.5**	19.6	19.8	19.8	**19.7**	13.7	13.3	0.8	**1.0**	-0.3	0.1		
			3	30.6	30.5	30.4	**30.5**	17.6	17.6	17.7	**17.6**	12.9	13.3	1.4	**1.0**	0.4	0.1	0.2	**0.3**
		glc	1	31.9	32.2	32.0	**32.0**	17.8	17.8	17.8	**17.8**	14.2	13.3	0.5	**0.5**	0.0	0.0		
			2	33.2	33.1	32.9	**33.1**	19.0	19.0	18.9	**19.0**	14.1	13.3	0.6	**0.5**	0.1	0.0		
			3	33.4	33.5	33.7	**33.5**	18.8	19.0	18.9	**18.9**	14.6	13.3	0.4	**0.5**	-0.1	0.0	0.0	**0.1**
		fru	1	30.7	30.2	30.3	**30.4**	16.7	16.7	16.7	**16.7**	13.7	13.3	0.8	**0.5**	0.3	0.1		
			2	31.2	31.0	32.5	**31.6**	17.2	17.3	17.2	**17.2**	14.3	13.3	0.5	**0.5**	0.0	0.0		
			3	31.7	31.0	31.4	**31.4**	16.0	15.9	15.4	**15.8**	15.6	13.3	0.2	**0.5**	-0.3	0.1	0.2	**0.3**
	2	suc	1	30.4	30.4	30.3	**30.4**	18.6	18.4	18.6	**18.5**	11.8	11.7	0.9	**1.0**	-0.1	0.0		
			2	29.4	29.3	29.4	**29.4**	17.7	17.6	17.5	**17.6**	11.8	11.7	0.9	**1.0**	-0.1	0.0		
			3	27.9	28.0	27.8	**27.9**	16.6	16.5	16.4	**16.5**	11.4	11.7	1.2	**1.0**	0.2	0.0	0.1	**0.2**
		glc	1	26.7	27.1	26.7	**26.8**	16.5	16.6	16.4	**16.5**	10.3	11.7	2.5	**2.3**	0.2	0.0		
			2	25.9	25.7	25.8	**25.8**	15.6	15.4	15.7	**15.6**	10.2	11.7	2.7	**2.3**	0.4	0.1		
			3	28.6	28.7	28.9	**28.7**	17.7	18.1	17.8	**17.9**	10.9	11.7	1.7	**2.3**	-0.6	0.3	0.5	**0.5**
		fru	1	26.9	26.7	26.8	**26.8**	15.6	15.6	15.5	**15.6**	11.2	11.7	1.4	**1.1**	0.3	0.1		
			2	29.6	29.2	29.4	**29.4**	17.7	17.5	17.4	**17.5**	11.9	11.7	0.9	**1.1**	-0.2	0.1		
			3	28.6	28.2	28.3	**28.4**	16.8	16.7	16.9	**16.8**	11.6	11.7	1.1	**1.1**	0.0	0.0	0.1	**0.2**
	3	suc	1	29.6	29.4	29.3	**29.4**	17.2	18.0	17.4	**17.5**	11.9	11.5	0.8	**1.0**	-0.3	0.1		
			2	24.2	24.2	24.6	**24.3**	13.2	13.0	13.2	**13.1**	11.2	11.5	1.2	**1.0**	0.2	0.0		
			3	24.3	24.5	24.6	**24.5**	13.1	13.1	13.1	**13.1**	11.4	11.5	1.1	**1.0**	0.1	0.0	0.1	**0.2**
		glc	1	28.2	28.2	28.3	**28.2**	15.9	15.8	16.0	**15.9**	12.3	11.5	0.6	**0.4**	0.1	0.0		
			2	31.2	31.4	31.4	**31.3**	18.7	18.7	18.5	**18.6**	12.7	11.5	0.4	**0.4**	0.0	0.0		
			3	29.9	29.8	30.0	**29.9**	16.8	16.8	16.8	**16.8**	13.1	11.5	0.3	**0.4**	-0.1	0.0	0.0	**0.1**
		fru	1	29.0	29.3	29.5	**29.3**	16.3	16.8	16.0	**16.4**	12.9	11.5	0.4	**0.4**	0.0	0.0		
			2	31.4	30.3	31.9	**31.2**	17.6	17.9	17.9	**17.8**	13.4	11.5	0.3	**0.4**	-0.1	0.0		
			3	30.8	30.9	31.2	**31.0**	18.5	18.5	18.6	**18.5**	12.4	11.5	0.5	**0.4**	0.1	0.0	0.0	**0.1**
	4	suc	1	29.1	29.4	28.8	**29.1**	16.8	17.3	17.2	**17.1**	12.0	11.6	0.8	**1.0**	-0.3	0.1		
			2	29.5	29.7	29.8	**29.7**	17.9	18.0	18.2	**18.0**	11.6	11.6	1.0	**1.0**	-0.1	0.0		
			3	25.6	25.8	25.7	**25.7**	14.5	14.5	14.7	**14.6**	11.1	11.6	1.4	**1.0**	0.3	0.1	0.2	**0.3**
		glc	1	26.1	26.0	26.6	**26.2**	14.7	14.5	14.8	**14.7**	11.6	11.6	1.0	**1.1**	-0.1	0.0		
			2	26.2	26.3	26.4	**26.3**	15.4	15.1	15.3	**15.3**	11.0	11.6	1.5	**1.1**	0.4	0.1		
			3	27.6	27.2	27.6	**27.5**	15.5	15.6	15.7	**15.6**	11.9	11.6	0.8	**1.1**	-0.3	0.1	0.2	**0.3**
		fru	1	28.9	29.3	29.6	**29.3**	16.0	16.1	16.4	**16.2**	13.1	11.6	0.4	**0.4**	-0.1	0.0		
			2	28.3	27.9	27.9	**28.0**	15.9	15.8	15.7	**15.8**	12.2	11.6	0.6	**0.4**	0.2	0.0		
			3	33.4	33.4	33.2	**33.3**	20.1	20.1	19.9	**20.0**	13.3	11.6	0.3	**0.4**	-0.1	0.0	0.1	**0.2**
	7	suc	1	28.5	28.3	28.2	**28.3**	15.5	15.8	16.1	**15.8**	12.5	11.9	0.6	**1.1**	-0.4	0.2		
			2	26.0	25.8	26.3	**26.0**	14.5	14.5	14.6	**14.5**	11.5	11.9	1.3	**1.1**	0.2	0.1		
			3	29.0	28.9	29.1	**29.0**	17.7	16.7	17.9	**17.4**	11.6	11.9	1.2	**1.1**	0.2	0.0	0.3	**0.4**
		glc	1	25.7	25.4	25.3	**25.5**	15.0	15.8	15.9	**15.6**	9.9	11.9	3.9	**3.4**	0.5	0.3		
			2	25.9	26.0	25.8	**25.9**	15.9	15.1	15.2	**15.4**	10.5	11.9	2.6	**3.4**	-0.8	0.6		
			3	27.5	27.4	27.8	**27.6**	17.7	17.3	17.7	**17.6**	10.0	11.9	3.6	**3.4**	0.3	0.1	1.0	**0.7**
		fru	1	27.3	27.6	27.3	**27.4**	15.6	15.2	14.8	**15.2**	12.2	11.9	0.8	**0.8**	0.0	0.0		
			2	28.9	28.6	28.7	**28.7**	14.7	15.4	15.6	**15.2**	13.5	11.9	0.3	**0.8**	-0.5	0.2		
			3	26.8	26.6	27.0	**26.8**	15.5	15.6	14.8	**15.3**	11.5	11.9	1.3	**0.8**	0.5	0.2	0.5	**0.5**
	14	suc	1	25.5	25.5	25.8	**25.6**	14.3	14.2	14.1	**14.2**	11.4	11.5	1.1	**1.0**	0.1	0.0		
			2	26.7	26.8	27.1	**26.9**	14.7	15.5	14.8	**15.0**	11.9	11.5	0.8	**1.0**	-0.2	0.1		
			3	26.3	26.3	26.9	**26.5**	14.9	15.0	15.7	**15.2**	11.3	11.5	1.2	**1.0**	0.2	0.0	0.1	**0.2**

		glc	1	25.9	25.7	26.2	**25.9**	14.7	14.6	14.7	**14.7**	11.3	11.5	1.2	**1.6**	-0.4	0.1		
			2	24.0	24.0	24.0	**24.0**	13.8	13.9	13.6	**13.8**	10.2	11.5	2.4	**1.6**	0.9	0.8		
			3	25.3	25.1	25.5	**25.3**	13.7	13.8	13.9	**13.8**	11.5	11.5	1.0	**1.6**	-0.5	0.3	1.2	**0.8**
		fru	1	26.5	26.5	26.4	**26.5**	14.8	14.8	15.1	**14.9**	11.6	11.5	1.0	**0.8**	0.2	0.0		
			2	26.4	26.2	26.5	**26.4**	14.7	14.5	14.4	**14.5**	11.8	11.5	0.8	**0.8**	0.1	0.0		
			3	27.7	27.1	26.8	**27.2**	14.6	14.7	14.6	**14.6**	12.6	11.5	0.5	**0.8**	-0.3	0.1	0.1	**0.2**

Gene	Day	Treatment	Sample	CT 1	CT 2	CT 3	Avg CT	18S CT 1	18S CT 2	18S CT3	Avg 18S CT	ΔCT	Avg suc ΔCT	Fold Change	Avg Fold Change	Deviation	Deviation^2	ΣDeviation^2	SD
FPS	1	suc	1	32.9	32.3	32.2	**32.5**	22.1	21.9	22.1	**22.0**	10.4	10.5	1.0	**1.0**	0.0	0.0		
			2	33.4	33.5	33.3	**33.4**	23.3	23.5	23.2	**23.3**	10.1	10.5	1.3	**1.0**	0.3	0.1		
			3	31.4	31.9	31.9	**31.7**	20.8	20.9	20.9	**20.9**	10.9	10.5	0.8	**1.0**	-0.3	0.1	0.2	**0.3**
		glc	1	32.5	32.7	33.0	**32.7**	25.0	25.6	24.8	**25.1**	7.6	10.5	7.2	**8.3**	-1.0	1.0		
			2	32.9	33.4	33.5	**33.3**	25.5	25.1	25.8	**25.5**	7.8	10.5	6.3	**8.3**	-2.0	3.8		
			3	30.8	31.0	31.7	**31.2**	24.0	24.1	24.5	**24.2**	7.0	10.5	11.2	**8.3**	3.0	8.8	13.7	**2.6**
		fru	1	30.4	30.3	30.1	**30.3**	20.4	20.0	20.4	**20.3**	10.0	10.5	1.4	**2.0**	-0.7	0.5		
			2	33.0	32.4	32.2	**32.5**	23.4	23.4	23.7	**23.5**	9.0	10.5	2.7	**2.0**	0.6	0.4		
			3	32.1	32.2	32.0	**32.1**	22.6	22.8	22.7	**22.7**	9.4	10.5	2.1	**2.0**	0.0	0.0	0.9	**0.7**
	2	suc	1	31.5	31.6	31.5	**31.5**	21.7	21.0	21.6	**21.4**	10.1	9.6	0.7	**1.0**	-0.3	0.1		
			2	30.2	30.4	30.4	**30.3**	21.1	20.9	21.6	**21.2**	9.1	9.6	1.4	**1.0**	0.4	0.1		
			3	32.1	32.2	32.6	**32.3**	22.2	23.0	22.6	**22.6**	9.7	9.6	1.0	**1.0**	-0.1	0.0	0.3	**0.4**
		glc	1	28.8	29.0	29.2	**29.0**	21.1	21.4	20.9	**21.1**	7.9	9.6	3.4	**3.7**	-0.3	0.1		
			2	27.2	26.9	27.1	**27.1**	19.5	19.6	19.6	**19.6**	7.5	9.6	4.4	**3.7**	0.7	0.5		
			3	32.3	32.2	32.4	**32.3**	24.4	24.2	24.5	**24.4**	7.9	9.6	3.3	**3.7**	-0.4	0.2	0.8	**0.6**
		fru	1	27.7	27.6	27.8	**27.7**	18.6	18.4	18.7	**18.6**	9.1	9.6	1.4	**0.9**	0.5	0.3		
			2	29.5	29.7	30.1	**29.8**	19.7	19.7	19.7	**19.7**	10.1	9.6	0.7	**0.9**	-0.1	0.0		
			3	29.6	29.5	29.6	**29.6**	18.9	18.9	19.1	**19.0**	10.6	9.6	0.5	**0.9**	-0.4	0.1	0.4	**0.5**
	3	suc	1	21.6	21.0	21.4	**21.3**	12.3	12.5	12.4	**12.4**	8.9	8.7	0.8	**1.1**	-0.2	0.0		
			2	20.7	20.6	20.9	**20.7**	11.5	11.7	11.8	**11.7**	9.1	8.7	0.8	**1.1**	-0.3	0.1		
			3	18.6	18.4	18.8	**18.6**	10.7	10.4	10.5	**10.5**	8.1	8.7	1.5	**1.1**	0.5	0.2	0.4	**0.4**
		glc	1	20.2	20.0	20.0	**20.1**	11.4	11.6	11.8	**11.6**	8.5	8.7	1.2	**1.2**	-0.1	0.0		
			2	21.8	21.9	21.0	**21.6**	13.0	12.8	12.5	**12.8**	8.8	8.7	0.9	**1.2**	-0.3	0.1		
			3	18.2	19.0	18.6	**18.6**	10.7	10.7	10.4	**10.6**	8.0	8.7	1.6	**1.2**	0.4	0.1	0.2	**0.3**
		fru	1	19.0	18.5	18.6	**18.7**	10.1	10.1	10.0	**10.1**	8.6	8.7	1.0	**0.8**	0.3	0.1		
			2	20.3	20.4	20.6	**20.4**	10.8	11.0	11.2	**11.0**	9.4	8.7	0.6	**0.8**	-0.2	0.0		
			3	21.0	20.8	20.9	**20.9**	11.8	11.5	11.4	**11.6**	9.3	8.7	0.6	**0.8**	-0.1	0.0	0.1	**0.2**
	4	suc	1	18.7	18.8	18.7	**18.7**	9.7	9.6	9.8	**9.7**	9.0	8.7	0.8	**1.0**	-0.2	0.1		
			2	19.0	19.2	19.4	**19.2**	10.9	10.7	11.0	**10.9**	8.3	8.7	1.3	**1.0**	0.3	0.1		
			3	17.3	17.6	17.9	**17.6**	8.7	9.0	9.1	**8.9**	8.7	8.7	1.0	**1.0**	0.0	0.0	0.1	**0.2**
		glc	1	18.6	18.3	18.4	**18.4**	10.1	9.8	10.0	**10.0**	8.5	8.7	1.2	**0.8**	0.4	0.2		
			2	19.3	19.5	19.1	**19.3**	10.1	10.0	9.9	**10.0**	9.3	8.7	0.6	**0.8**	-0.1	0.0		
			3	20.0	19.9	19.8	**19.9**	10.1	10.1	10.3	**10.2**	9.7	8.7	0.5	**0.8**	-0.3	0.1	0.2	**0.4**
		fru	1	22.4	20.9	21.8	**21.7**	12.5	12.4	13.0	**12.6**	9.1	8.7	0.8	**0.8**	0.0	0.0		
			2	18.9	18.6	19.2	**18.9**	10.0	10.2	10.3	**10.2**	8.7	8.7	1.0	**0.8**	0.1	0.0		
			3	21.1	21.1	21.5	**21.2**	12.0	12.1	12.1	**12.1**	9.2	8.7	0.7	**0.8**	-0.1	0.0	0.0	**0.1**
	7	suc	1	26.6	26.4	26.4	**26.5**	16.7	16.8	16.7	**16.7**	9.7	9.3	0.7	**1.0**	-0.3	0.1		
			2	24.7	24.8	24.8	**24.8**	16.1	16.1	16.0	**16.1**	8.7	9.3	1.5	**1.0**	0.5	0.2		
			3	28.0	27.9	27.9	**27.9**	18.5	18.3	18.6	**18.5**	9.5	9.3	0.9	**1.0**	-0.2	0.0	0.3	**0.4**
		glc	1	23.6	23.7	24.0	**23.8**	14.9	15.1	15.2	**15.1**	8.7	9.3	1.5	**1.7**	-0.2	0.0		
			2	22.8	22.6	22.7	**22.7**	14.0	13.8	13.8	**13.9**	8.8	9.3	1.4	**1.7**	-0.3	0.1		
			3	28.1	28.0	28.1	**28.1**	19.9	19.9	19.8	**19.9**	8.2	9.3	2.1	**1.7**	0.5	0.2	0.3	**0.4**
		fru	1	23.6	23.6	23.5	**23.6**	14.1	14.2	14.1	**14.1**	9.4	9.3	0.9	**0.9**	0.0	0.0		
			2	23.7	23.8	24.3	**23.9**	14.2	14.3	14.8	**14.4**	9.5	9.3	0.9	**0.9**	0.0	0.0		
			3	23.9	23.7	23.8	**23.8**	14.5	14.4	14.4	**14.4**	9.4	9.3	1.0	**0.9**	0.0	0.0	0.0	**0.0**
	14	suc	1	24.0	24.2	24.0	**24.1**	15.7	15.7	15.6	**15.7**	8.4	8.9	1.4	**1.1**	0.3	0.1		

			2	22.9	23.1	23.1	**23.0**	14.6	14.6	14.6	**14.6**	8.4	8.9	1.4	**1.1**	0.3	0.1		
			3	24.7	24.8	25.1	**24.9**	14.9	14.9	15.2	**15.0**	9.9	8.9	0.5	**1.1**	-0.6	0.3	0.5	**0.5**
		glc	1	22.5	22.2	22.2	**22.3**	14.2	14.6	14.2	**14.3**	8.0	8.9	1.9	**1.4**	0.5	0.3		
			2	23.1	23.0	23.0	**23.0**	14.9	14.9	14.8	**14.9**	8.2	8.9	1.7	**1.4**	0.3	0.1		
			3	22.5	22.4	22.7	**22.5**	12.8	12.8	12.9	**12.8**	9.7	8.9	0.6	**1.4**	-0.8	0.7	1.0	**0.7**
		fru	1	21.8	21.9	22.2	**22.0**	14.0	14.1	14.3	**14.1**	7.8	8.9	2.1	**1.5**	0.6	0.4		
			2	22.9	23.0	22.8	**22.9**	14.7	14.6	14.6	**14.6**	8.3	8.9	1.6	**1.5**	0.1	0.0		
			3	24.5	24.6	24.7	**24.6**	15.1	15.3	15.3	**15.2**	9.4	8.9	0.7	**1.5**	-0.7	0.5	1.0	**0.7**

Gene	Day	Treatment	Sample	CT 1	CT 2	CT 3	Avg CT	18S CT 1	18S CT 2	18S CT3	Avg 18S CT	ΔCT	Avg suc ΔCT	Fold Change	Avg Fold Change	Deviation	$Deviation^2$	$\Sigma Deviation^2$	SD
HMGR	1	suc	1	30.9	31.4	31.6	**31.3**	22.1	21.9	22.1	**22.0**	9.3	8.5	0.6	**1.1**	-0.5	0.2		
			2	31.4	31.1	31.1	**31.2**	23.3	23.5	23.2	**23.3**	7.9	8.5	1.6	**1.1**	0.5	0.3		
			3	28.8	29.7	29.5	**29.3**	20.8	20.9	20.9	**20.9**	8.5	8.5	1.0	**1.1**	0.0	0.0	0.5	**0.5**
		glc	1	31.6	31.6	31.1	**31.4**	25.0	25.6	24.8	**25.1**	6.3	8.5	4.7	**3.6**	1.1	1.2		
			2	32.2	33.3	32.0	**32.5**	25.5	25.1	25.8	**25.5**	7.0	8.5	2.8	**3.6**	-0.8	0.6		
			3	30.7	31.1	31.2	**31.0**	24.0	24.1	24.5	**24.2**	6.8	8.5	3.3	**3.6**	-0.3	0.1	1.9	**1.0**
		fru	1	27.7	28.0	27.7	**27.8**	20.4	20.0	20.4	**20.3**	7.5	8.5	2.0	**2.5**	-0.5	0.2		
			2	30.9	30.5	30.7	**30.7**	23.4	23.4	23.7	**23.5**	7.2	8.5	2.5	**2.5**	0.0	0.0		
			3	29.8	29.7	29.6	**29.7**	22.6	22.8	22.7	**22.7**	7.0	8.5	2.9	**2.5**	0.4	0.2	0.4	**0.4**
	2	suc	1	29.3	30.5	29.7	**29.8**	21.7	21.0	21.6	**21.4**	8.4	7.8	0.7	**1.0**	-0.4	0.2		
			2	28.7	29.2	28.8	**28.9**	21.1	20.9	21.6	**21.2**	7.7	7.8	1.1	**1.0**	0.0	0.0		
			3	29.8	30.1	29.8	**29.9**	22.2	23.0	22.6	**22.6**	7.3	7.8	1.4	**1.0**	0.4	0.1	0.3	**0.4**
		glc	1	30.0	29.9	29.5	**29.8**	21.1	21.4	20.9	**21.1**	8.7	7.8	0.5	**0.6**	-0.1	0.0		
			2	29.0	28.9	29.2	**29.0**	19.5	19.6	19.6	**19.6**	9.5	7.8	0.3	**0.6**	-0.3	0.1		
			3	32.0	32.4	32.0	**32.1**	24.4	24.2	24.5	**24.4**	7.8	7.8	1.0	**0.6**	0.4	0.2	0.3	**0.4**
		fru	1	28.1	28.1	28.5	**28.2**	18.6	18.4	18.7	**18.6**	9.7	7.8	0.3	**0.3**	0.0	0.0		
			2	28.8	28.5	28.8	**28.7**	19.7	19.7	19.7	**19.7**	9.0	7.8	0.4	**0.3**	0.1	0.0		
			3	28.6	28.8	29.1	**28.8**	18.9	18.9	19.1	**19.0**	9.9	7.8	0.2	**0.3**	-0.1	0.0	0.0	**0.1**
	3	suc	1	21.2	20.5	21.0	**20.9**	12.3	12.5	12.4	**12.4**	8.5	7.9	0.7	**1.1**	-0.4	0.2		
			2	19.8	19.9	19.7	**19.8**	11.5	11.7	11.8	**11.7**	8.1	7.9	0.9	**1.1**	-0.2	0.1		
			3	17.2	18.0	17.6	**17.6**	10.7	10.4	10.5	**10.5**	7.1	7.9	1.8	**1.1**	0.7	0.5	0.7	**0.6**
		glc	1	19.8	19.8	19.5	**19.7**	11.4	11.6	11.8	**11.6**	8.1	7.9	0.9	**1.0**	-0.1	0.0		
			2	20.3	20.4	20.2	**20.3**	13.0	12.8	12.5	**12.8**	7.5	7.9	1.3	**1.0**	0.3	0.1		
			3	18.4	19.1	18.7	**18.7**	10.7	10.7	10.4	**10.6**	8.1	7.9	0.9	**1.0**	-0.2	0.0	0.1	**0.2**
		fru	1	19.5	19.5	19.6	**19.5**	10.1	10.1	10.0	**10.1**	9.5	7.9	0.3	**0.6**	-0.2	0.1		
			2	19.3	19.2	19.0	**19.2**	10.8	11.0	11.2	**11.0**	8.2	7.9	0.8	**0.6**	0.3	0.1		
			3	20.7	20.3	20.0	**20.3**	11.8	11.5	11.4	**11.6**	8.8	7.9	0.5	**0.6**	0.0	0.0	0.1	**0.2**
	4	suc	1	18.4	18.0	18.2	**18.2**	9.7	9.6	9.8	**9.7**	8.5	7.7	0.6	**1.1**	-0.5	0.3		
			2	17.8	17.7	17.6	**17.7**	10.9	10.7	11.0	**10.9**	6.8	7.7	1.8	**1.1**	0.7	0.5		
			3	16.5	16.8	16.9	**16.7**	8.7	9.0	9.1	**8.9**	7.8	7.7	0.9	**1.1**	-0.2	0.0	0.8	**0.6**
		glc	1	17.6	17.6	17.8	**17.7**	10.1	9.8	10.0	**10.0**	7.7	7.7	1.0	**0.7**	0.3	0.1		
			2	17.8	17.9	18.0	**17.9**	10.1	10.0	9.9	**10.0**	7.9	7.7	0.9	**0.7**	0.2	0.0		
			3	19.8	19.6	19.7	**19.7**	10.1	10.1	10.3	**10.2**	9.5	7.7	0.3	**0.7**	-0.4	0.2	0.3	**0.4**
		fru	1	20.5	21.0	20.7	**20.7**	12.5	12.4	13.0	**12.6**	8.1	7.7	0.8	**0.5**	0.3	0.1		
			2	19.1	19.7	19.6	**19.5**	10.0	10.2	10.3	**10.2**	9.3	7.7	0.3	**0.5**	-0.1	0.0		
			3	21.0	21.5	21.4	**21.3**	12.0	12.1	12.1	**12.1**	9.2	7.7	0.3	**0.5**	-0.1	0.0	0.1	**0.2**
	7	suc	1	25.0	25.0	25.3	**25.1**	16.7	16.8	16.7	**16.7**	8.4	8.3	0.9	**1.1**	-0.1	0.0		
			2	23.6	23.7	24.0	**23.8**	16.1	16.1	16.0	**16.1**	7.7	8.3	1.5	**1.1**	0.5	0.2		
			3	27.4	27.0	27.4	**27.3**	18.5	18.3	18.6	**18.5**	8.8	8.3	0.7	**1.1**	-0.3	0.1	0.3	**0.4**
		glc	1	23.7	23.9	23.2	**23.6**	14.9	15.1	15.2	**15.1**	8.5	8.3	0.8	**1.0**	-0.1	0.0		
			2	21.8	21.8	21.3	**21.6**	14.0	13.8	13.8	**13.9**	7.8	8.3	1.4	**1.0**	0.5	0.2		
			3	29.3	28.8	28.4	**28.8**	19.9	19.9	19.8	**19.9**	9.0	8.3	0.6	**1.0**	-0.3	0.1	0.4	**0.4**
		fru	1	23.0	23.0	22.7	**22.9**	14.1	14.2	14.1	**14.1**	8.8	8.3	0.7	**0.9**	-0.1	0.0		
			2	22.8	22.8	22.5	**22.7**	14.2	14.3	14.8	**14.4**	8.3	8.3	1.0	**0.9**	0.2	0.0		

			3	23.1	23.1	22.8	23.0	14.5	14.4	14.4	14.4	8.6	8.3	0.8	0.9	0.0	0.0	0.0	0.2
	14	suc	1	23.9	24.0	24.0	24.0	15.7	15.7	15.6	15.7	8.3	8.2	0.9	1.1	-0.2	0.0		
			2	22.2	22.1	22.0	22.1	14.6	14.6	14.6	14.6	7.5	8.2	1.6	1.1	0.5	0.3		
			3	23.7	23.7	23.6	23.7	14.9	14.9	15.2	15.0	8.7	8.2	0.7	1.1	-0.4	0.1	0.4	0.5
		glc	1	22.0	22.0	22.1	22.0	14.2	14.6	14.2	14.3	7.7	8.2	1.4	0.8	0.5	0.3		
			2	23.7	23.8	23.8	23.8	14.9	14.9	14.8	14.9	8.9	8.2	0.6	0.8	-0.2	0.1		
			3	21.8	22.0	21.9	21.9	12.8	12.8	12.9	12.8	9.1	8.2	0.5	0.8	-0.3	0.1	0.4	0.5
		fru	1	21.8	21.7	21.8	21.8	14.0	14.1	14.3	14.1	7.6	8.2	1.4	0.7	0.7	0.5		
			2	23.6	23.7	23.7	23.7	14.7	14.6	14.6	14.6	9.0	8.2	0.5	0.7	-0.2	0.0		
			3	25.9	26.1	25.4	25.8	15.1	15.3	15.3	15.2	10.6	8.2	0.2	0.7	-0.5	0.3	0.8	0.6

Gene	Day	Treatment	Sample	CT 1	CT 2	CT 3	Avg CT	18S CT 1	18S CT 2	18S CT3	Avg 18S CT	ΔCT	Avg suc ΔCT	Fold Change	Avg Fold Change	Deviation	$Deviation^2$	$ΣDeviation^2$	SD
DXR	1	suc	1	32.3	32.9	32.3	32.5	22.1	21.9	22.1	22.0	10.5	11.1	1.5	1.1	0.5	0.2		
			2	34.3	34.5	34.2	34.3	23.3	23.5	23.2	23.3	11.0	11.1	1.1	1.1	0.0	0.0		
			3	33.1	32.5	32.4	32.7	20.8	20.9	20.9	20.9	11.8	11.1	0.6	1.1	-0.5	0.2	0.4	0.5
		glc	1	33.1	33.4	33.0	33.2	25.0	25.6	24.8	25.1	8.0	11.1	8.3	8.0	0.3	0.1		
			2	32.9	33.0	33.5	33.1	25.5	25.1	25.8	25.5	7.7	11.1	10.7	8.0	2.7	7.5		
			3	32.8	33.1	33.1	33.0	24.0	24.1	24.5	24.2	8.8	11.1	4.9	8.0	-3.1	9.5	17.2	2.9
		fru	1	30.9	31.5	30.7	31.0	20.4	20.0	20.4	20.3	10.8	11.1	1.3	2.6	-1.3	1.7		
			2	33.0	32.1	33.1	32.7	23.4	23.4	23.7	23.5	9.2	11.1	3.6	2.6	1.1	1.1		
			3	32.0	32.6	32.3	32.3	22.6	22.8	22.7	22.7	9.6	11.1	2.8	2.6	0.2	0.1	2.9	1.2
	2	suc	1	31.7	31.8	32.0	31.8	21.7	21.0	21.6	21.4	10.4	10.1	0.8	1.0	-0.2	0.0		
			2	30.7	31.1	30.7	30.8	21.1	20.9	21.6	21.2	9.6	10.1	1.4	1.0	0.4	0.1		
			3	32.8	33.2	32.7	32.9	22.2	23.0	22.6	22.6	10.3	10.1	0.9	1.0	-0.2	0.0	0.2	0.3
		glc	1	30.2	30.5	30.8	30.5	21.1	21.4	20.9	21.1	9.4	10.1	1.7	2.0	-0.3	0.1		
			2	28.5	28.7	28.7	28.6	19.5	19.6	19.6	19.6	9.1	10.1	2.1	2.0	0.1	0.0		
			3	33.2	33.9	33.0	33.4	24.4	24.2	24.5	24.4	9.0	10.1	2.2	2.0	0.2	0.0	0.1	0.3
		fru	1	28.3	28.1	28.1	28.2	18.6	18.4	18.7	18.6	9.6	10.1	1.4	0.9	0.5	0.3		
			2	29.7	30.1	30.0	29.9	19.7	19.7	19.7	19.7	10.2	10.1	0.9	0.9	0.0	0.0		
			3	30.0	30.1	30.9	30.3	18.9	18.9	19.1	19.0	11.4	10.1	0.4	0.9	-0.5	0.3	0.5	0.5
	3	suc	1	20.2	20.4	20.5	20.4	8.9	9.2	9.5	9.2	11.2	10.1	0.5	1.2	-0.7	0.5		
			2	19.6	19.4	19.3	19.4	10.0	10.6	10.4	10.3	9.1	10.1	1.9	1.2	0.8	0.6		
			3	21.7	21.6	21.8	21.7	11.7	11.8	11.9	11.8	9.9	10.1	1.1	1.2	-0.1	0.0	1.1	0.7
		glc	1	24.7	24.2	24.6	24.5	16.4	16.2	16.4	16.3	8.2	10.1	3.7	3.0	0.7	0.5		
			2	25.4	25.5	25.9	25.6	16.1	16.1	16.2	16.1	9.5	10.1	1.5	3.0	-1.5	2.3		
			3	23.9	23.6	23.6	23.7	15.0	15.9	15.9	15.6	8.1	10.1	3.9	3.0	0.8	0.7	3.5	1.3
		fru	1	23.3	23.4	23.5	23.4	13.4	13.3	13.3	13.3	10.1	10.1	1.0	0.8	0.2	0.0		
			2	24.2	24.3	24.4	24.3	13.8	13.9	13.8	13.8	10.5	10.1	0.8	0.8	0.0	0.0		
			3	24.1	23.9	24.5	24.2	13.1	13.6	13.6	13.4	10.7	10.1	0.6	0.8	-0.2	0.0	0.1	0.2
	4	suc	1	22.0	21.8	21.7	21.8	11.3	11.0	10.9	11.1	10.8	9.8	0.5	1.1	-0.6	0.3		
			2	22.1	21.7	21.6	21.8	12.6	12.4	12.8	12.6	9.2	9.8	1.5	1.1	0.4	0.2		
			3	19.5	19.5	19.5	19.5	10.0	10.0	10.1	10.0	9.5	9.8	1.3	1.1	0.2	0.0	0.6	0.5
		glc	1	21.7	21.8	21.8	21.8	13.0	12.9	13.2	13.0	8.7	9.8	2.1	1.4	0.7	0.5		
			2	22.4	22.6	22.7	22.6	12.9	12.6	12.7	12.7	9.8	9.8	1.0	1.4	-0.4	0.2		
			3	22.4	22.2	22.4	22.3	12.2	12.8	12.9	12.6	9.7	9.8	1.1	1.4	-0.3	0.1	0.8	0.6
		fru	1	21.9	22.0	21.8	21.9	11.3	11.4	11.4	11.4	10.5	9.8	0.6	0.5	0.1	0.0		
			2	21.1	21.7	21.2	21.3	10.8	10.2	10.2	10.4	10.9	9.8	0.5	0.5	0.0	0.0		
			3	25.2	25.2	25.6	25.3	14.0	13.9	14.1	14.0	11.3	9.8	0.3	0.5	-0.1	0.0	0.0	0.1
	7	suc	1	24.6	24.3	24.1	24.3	14.0	14.1	13.9	14.0	10.3	10.0	0.8	1.0	-0.2	0.1		
			2	23.5	23.6	23.7	23.6	13.9	14.0	14.1	14.0	9.6	10.0	1.3	1.0	0.3	0.1		
			3	26.5	26.4	26.9	26.6	16.3	16.8	16.7	16.6	10.0	10.0	1.0	1.0	0.0	0.0	0.1	0.3
		glc	1	23.5	23.8	23.7	23.7	13.5	13.6	13.9	13.7	10.0	10.0	1.0	1.5	-0.6	0.3		
			2	22.4	22.6	22.3	22.4	14.0	14.0	13.9	14.0	8.5	10.0	2.9	1.5	1.3	1.7		

		fru	1	23.6	23.7	23.9	**23.7**	13.7	13.8	13.8	**13.8**	10.0	10.0	1.0	**0.8**	0.2	0.0		
			2	24.5	24.7	24.8	**24.7**	14.2	14.4	14.7	**14.4**	10.2	10.0	0.8	**0.8**	0.0	0.0		
			3	25.6	25.7	25.1	**25.5**	14.7	14.8	15.0	**14.8**	10.6	10.0	0.6	**0.8**	-0.2	0.0	0.1	**0.2**
	14	suc	1	24.7	24.3	24.4	**24.5**	13.9	13.9	13.8	**13.9**	10.6	10.3	0.8	**1.0**	-0.2	0.1		
			2	23.1	23.0	22.9	**23.0**	13.3	13.1	13.2	**13.2**	9.8	10.3	1.4	**1.0**	0.3	0.1		
			3	24.7	24.4	24.7	**24.6**	14.3	14.3	14.1	**14.2**	10.4	10.3	0.9	**1.0**	-0.1	0.0	0.2	**0.3**
		glc	1	22.7	22.7	23.0	**22.8**	13.5	13.7	13.9	**13.7**	9.1	10.3	2.2	**2.0**	0.2	0.0		
			2	22.3	21.9	22.3	**22.2**	13.6	13.4	13.4	**13.5**	8.7	10.3	2.9	**2.0**	0.9	0.8		
			3	22.8	23.0	22.8	**22.9**	12.5	12.6	12.5	**12.5**	10.3	10.3	0.9	**2.0**	-1.1	1.2	2.0	**1.0**
		fru	1	23.3	23.2	23.2	**23.2**	13.5	13.5	13.2	**13.4**	9.8	10.3	1.3	**1.2**	0.1	0.0		
			2	23.7	23.8	23.8	**23.8**	13.9	14.2	14.1	**14.1**	9.7	10.3	1.5	**1.2**	0.2	0.1		
			3	22.8	22.9	22.6	**22.8**	12.8	12.2	12.0	**12.3**	10.4	10.3	0.9	**1.2**	-0.3	0.1	0.2	**0.3**

Gene	Day	Treatment	Sample	CT 1	CT 2	CT 3	Avg CT	18S CT 1	18S CT 2	18S CT3	Avg 18S CT	ΔCT	Avg suc ΔCT	Fold Change	Avg Fold Change	Deviation	Deviation^2	ΣDeviation^2	SD
DXS	1	suc	1	32.1	33.1	32.7	**32.6**	22.1	21.9	22.1	**22.0**	10.6	11.0	1.3	**1.0**	0.3	0.1		
			2	34.2	33.6	34.8	**34.2**	23.3	23.5	23.2	**23.3**	10.9	11.0	1.1	**1.0**	0.0	0.0		
			3	31.8	33.1	32.0	**32.3**	20.8	20.9	20.9	**20.9**	11.4	11.0	0.7	**1.0**	-0.3	0.1	0.2	**0.3**
		glc	1	35.3	33.1	34.0	**34.1**	25.0	25.6	24.8	**25.1**	9.0	11.0	3.9	**5.2**	-1.3	1.8		
			2	34.9	34.1	33.2	**34.1**	25.5	25.1	25.8	**25.5**	8.6	11.0	5.2	**5.2**	-0.1	0.0		
			3	32.6	32.3	32.4	**32.4**	24.0	24.1	24.5	**24.2**	8.2	11.0	6.6	**5.2**	1.4	2.0	3.8	**1.4**
		fru	1	31.1	30.3	29.9	**30.4**	20.4	20.0	20.4	**20.3**	10.2	11.0	1.7	**1.7**	0.0	0.0		
			2	34.8	32.7	35.0	**34.2**	23.4	23.4	23.7	**23.5**	10.7	11.0	1.2	**1.7**	-0.5	0.3		
			3	32.2	32.5	32.8	**32.5**	22.6	22.8	22.7	**22.7**	9.8	11.0	2.2	**1.7**	0.5	0.3	0.5	**0.5**
	2	suc	1	32.4	32.0	33.1	**32.5**	21.7	21.0	21.6	**21.4**	11.1	10.6	0.7	**1.0**	-0.3	0.1		
			2	31.8	30.9	32.0	**31.6**	21.1	20.9	21.6	**21.2**	10.4	10.6	1.2	**1.0**	0.2	0.0		
			3	33.2	33.0	32.7	**33.0**	22.2	23.0	22.6	**22.6**	10.4	10.6	1.2	**1.0**	0.2	0.0	0.1	**0.3**
		glc	1	29.4	29.8	29.4	**29.5**	21.1	21.4	20.9	**21.1**	8.4	10.6	4.6	**8.4**	-3.8	14.2		
			2	26.4	27.2	27.3	**27.0**	19.5	19.6	19.6	**19.6**	7.4	10.6	9.2	**8.4**	0.8	0.7		
			3	31.3	31.5	31.6	**31.5**	24.4	24.2	24.5	**24.4**	7.1	10.6	11.3	**8.4**	2.9	8.7	23.6	**3.4**
		fru	1	29.7	29.9	30.0	**29.9**	18.6	18.4	18.7	**18.6**	11.3	10.6	0.6	**1.0**	-0.4	0.2		
			2	29.8	29.6	30.2	**29.9**	19.7	19.7	19.7	**19.7**	10.2	10.6	1.4	**1.0**	0.3	0.1		
			3	29.0	29.6	29.6	**29.4**	18.9	18.9	19.1	**19.0**	10.4	10.6	1.1	**1.0**	0.1	0.0	0.3	**0.4**
	3	suc	1	19.2	19.8	19.5	**19.5**	8.9	9.2	9.5	**9.2**	10.3	10.1	0.9	**1.0**	-0.1	0.0		
			2	20.3	20.1	20.2	**20.2**	10.0	10.6	10.4	**10.3**	9.9	10.1	1.2	**1.0**	0.2	0.0		
			3	22.1	22.7	21.3	**22.0**	11.7	11.8	11.9	**11.8**	10.2	10.1	0.9	**1.0**	-0.1	0.0	0.1	**0.2**
		glc	1	24.8	24.7	24.6	**24.7**	16.4	16.2	16.4	**16.3**	8.4	10.1	3.4	**2.6**	0.8	0.6		
			2	25.7	25.9	25.5	**25.7**	16.1	16.1	16.2	**16.1**	9.6	10.1	1.5	**2.6**	-1.2	1.3		
			3	24.2	24.0	24.2	**24.1**	15.0	15.9	15.9	**15.6**	8.5	10.1	3.0	**2.6**	0.4	0.2	2.1	**1.0**
		fru	1	23.5	23.1	23.1	**23.2**	13.4	13.3	13.3	**13.3**	9.9	10.1	1.2	**0.9**	0.2	0.1		
			2	23.6	24.7	24.3	**24.2**	13.8	13.9	13.8	**13.8**	10.4	10.1	0.9	**0.9**	-0.1	0.0		
			3	23.8	23.9	24.0	**23.9**	13.1	13.6	13.6	**13.4**	10.5	10.1	0.8	**0.9**	-0.1	0.0	0.1	**0.2**
	4	suc	1	22.2	22.0	22.3	**22.2**	11.3	11.0	10.9	**11.1**	11.1	10.4	0.6	**1.1**	-0.5	0.2		
			2	22.7	22.4	22.3	**22.5**	12.6	12.4	12.8	**12.6**	9.9	10.4	1.4	**1.1**	0.4	0.1		
			3	20.7	19.8	20.0	**20.2**	10.0	10.0	10.1	**10.0**	10.1	10.4	1.2	**1.1**	0.1	0.0	0.3	**0.4**
		glc	1	22.6	22.7	22.2	**22.5**	13.0	12.9	13.2	**13.0**	9.5	10.4	1.9	**1.2**	0.6	0.4		
			2	22.3	22.5	22.3	**22.4**	12.9	12.6	12.7	**12.7**	9.6	10.4	1.7	**1.2**	0.4	0.2		
			3	25.6	25.4	25.4	**25.5**	12.2	12.8	12.9	**12.6**	12.8	10.4	0.2	**1.2**	-1.1	1.1	1.7	**0.9**
		fru	1	22.0	21.9	21.8	**21.9**	11.3	11.4	11.4	**11.4**	10.5	10.4	0.9	**0.5**	0.3	0.1		
			2	21.5	21.8	21.5	**21.6**	10.8	10.2	10.2	**10.4**	11.2	10.4	0.6	**0.5**	0.0	0.0		
			3	26.8	26.8	26.7	**26.8**	14.0	13.9	14.1	**14.0**	12.8	10.4	0.2	**0.5**	-0.4	0.1	0.2	**0.4**
	7	suc	1	24.8	24.6	24.6	**24.7**	14.0	14.1	13.9	**14.0**	10.7	10.4	0.8	**1.0**	-0.2	0.0		
			2	23.9	24.0	24.1	**24.0**	13.9	14.0	14.1	**14.0**	10.0	10.4	1.3	**1.0**	0.3	0.1		
			3	26.9	27.2	27.1	**27.1**	16.3	16.8	16.7	**16.6**	10.5	10.4	0.9	**1.0**	-0.1	0.0	0.1	**0.2**

			2	23.4	23.2	23.3	**23.3**	14.0	14.0	13.9	**14.0**	9.3	10.4	2.1	**1.1**	1.0	1.0		
			3	28.6	28.7	28.7	**28.7**	17.2	17.2	17.4	**17.3**	11.4	10.4	0.5	**1.1**	-0.6	0.3	1.5	**0.9**
		fru	1	24.2	24.3	24.3	**24.3**	13.7	13.8	13.8	**13.8**	10.5	10.4	0.9	**0.8**	0.1	0.0		
			2	24.9	25.2	25.0	**25.0**	14.2	14.4	14.7	**14.4**	10.6	10.4	0.9	**0.8**	0.0	0.0		
			3	25.7	25.8	26.0	**25.8**	14.7	14.8	15.0	**14.8**	11.0	10.4	0.6	**0.8**	-0.2	0.0	0.0	**0.1**
	14	suc	1	24.4	24.5	24.1	**24.3**	13.9	13.9	13.8	**13.9**	10.5	10.1	0.8	**1.0**	-0.2	0.1		
			2	23.3	23.2	23.1	**23.2**	13.3	13.1	13.2	**13.2**	10.0	10.1	1.1	**1.0**	0.1	0.0		
			3	24.0	24.2	24.2	**24.1**	14.3	14.3	14.1	**14.2**	9.9	10.1	1.2	**1.0**	0.2	0.0	0.1	**0.2**
		glc	1	22.8	23.1	23.0	**23.0**	13.5	13.7	13.9	**13.7**	9.3	10.1	1.8	**1.7**	0.1	0.0		
			2	22.7	22.4	22.3	**22.5**	13.6	13.4	13.4	**13.5**	9.0	10.1	2.2	**1.7**	0.5	0.3		
			3	22.6	22.8	22.4	**22.6**	12.5	12.6	12.5	**12.5**	10.1	10.1	1.0	**1.7**	-0.6	0.4	0.7	**0.6**
		fru	1	23.6	23.9	23.8	**23.8**	13.5	13.5	13.2	**13.4**	10.4	10.1	0.8	**0.7**	0.2	0.0		
			2	24.6	25.1	25.2	**25.0**	13.9	14.2	14.1	**14.1**	10.9	10.1	0.6	**0.7**	-0.1	0.0		
			3	23.3	23.5	23.0	**23.3**	12.8	12.2	12.0	**12.3**	10.9	10.1	0.6	**0.7**	-0.1	0.0	0.0	**0.2**

Gene	Day	Treatment	Sample	CT 1	CT 2	CT 3	Avg CT	18S CT 1	18S CT 2	18S CT3	Avg 18S CT	ΔCT	Avg suc ΔCT	Fold Change	Avg Fold Change	Deviation	Deviation^2	ΣDeviation^2	SD
SQS	1	suc	1	31.0	31.2	31.3	**31.2**	19.3	19.1	19.4	**19.3**	11.9	11.6	0.8	**1.7**	-0.8	0.7		
			2	29.4	29.5	29.4	**29.4**	19.6	19.8	19.8	**19.7**	9.7	11.6	3.8	**1.7**	2.2	4.8		
			3	30.6	31.3	31.0	**31.0**	17.6	17.6	17.7	**17.6**	13.3	11.6	0.3	**1.7**	-1.4	1.8	7.3	**1.9**
		glc	1	26.9	27.7	27.2	**27.3**	17.8	17.8	17.8	**17.8**	9.5	11.6	4.5	**3.6**	1.0	0.9		
			2	29.0	28.9	29.0	**29.0**	19.0	19.0	18.9	**19.0**	10.0	11.6	3.1	**3.6**	-0.4	0.2		
			3	28.9	29.0	28.9	**28.9**	18.8	19.0	18.9	**18.9**	10.0	11.6	3.1	**3.6**	-0.5	0.3	1.4	**0.8**
		fru	1	28.9	29.3	29.1	**29.1**	16.7	16.7	16.7	**16.7**	12.4	11.6	0.6	**0.7**	-0.1	0.0		
			2	29.1	29.5	29.3	**29.3**	17.2	17.3	17.2	**17.2**	12.1	11.6	0.7	**0.7**	0.1	0.0		
			3	27.9	28.3	28.1	**28.1**	16.0	15.9	15.4	**15.8**	12.3	11.6	0.6	**0.7**	0.0	0.0	0.0	**0.1**
	2	suc	1	28.5	28.3	28.4	**28.4**	18.6	18.4	18.6	**18.5**	9.9	10.1	1.1	**1.0**	0.1	0.0		
			2	28.1	27.5	27.8	**27.8**	17.7	17.6	17.5	**17.6**	10.2	10.1	0.9	**1.0**	-0.1	0.0		
			3	26.6	26.6	26.6	**26.6**	16.6	16.5	16.4	**16.5**	10.1	10.1	1.0	**1.0**	0.0	0.0	0.0	**0.1**
		glc	1	26.2	26.3	26.3	**26.3**	16.5	16.6	16.4	**16.5**	9.8	10.1	1.2	**1.0**	0.3	0.1		
			2	26.0	26.6	26.3	**26.3**	15.6	15.4	15.7	**15.6**	10.7	10.1	0.6	**1.0**	-0.3	0.1		
			3	27.8	27.9	27.8	**27.8**	17.7	18.1	17.8	**17.9**	10.0	10.1	1.1	**1.0**	0.1	0.0	0.2	**0.3**
		fru	1	26.4	27.0	26.7	**26.7**	15.6	15.6	15.5	**15.6**	11.1	10.1	0.5	**1.5**	-1.0	1.0		
			2	26.0	26.2	26.1	**26.1**	17.7	17.5	17.4	**17.5**	8.6	10.1	2.8	**1.5**	1.3	1.8		
			3	26.6	26.7	26.7	**26.7**	16.8	16.7	16.9	**16.8**	9.9	10.1	1.1	**1.5**	-0.3	0.1	2.9	**1.2**
	3	suc	1	32.1	31.9	32.3	**32.1**	21.2	21.4	21.8	**21.5**	10.6	10.2	0.7	**1.2**	-0.4	0.2		
			2	23.6	23.7	23.7	**23.7**	12.9	12.7	13.1	**12.9**	10.8	10.2	0.7	**1.2**	-0.5	0.3		
			3	25.6	25.1	25.4	**25.4**	16.3	16.3	16.3	**16.3**	9.1	10.2	2.1	**1.2**	1.0	0.9	1.4	**0.8**
		glc	1	30.8	30.8	31.3	**31.0**	18.8	18.9	18.8	**18.8**	12.1	10.2	0.3	**0.4**	-0.2	0.0		
			2	32.4	32.6	32.9	**32.6**	21.6	22.0	21.5	**21.7**	10.9	10.2	0.6	**0.4**	0.2	0.0		
			3	31.7	31.7	31.1	**31.5**	20.1	19.9	20.6	**20.2**	11.3	10.2	0.5	**0.4**	0.0	0.0	0.1	**0.2**
		fru	1	27.7	27.8	27.9	**27.8**	17.0	16.9	17.1	**17.0**	10.8	10.2	0.6	**0.4**	0.3	0.1		
			2	31.6	32.2	31.9	**31.9**	18.9	18.8	18.9	**18.9**	13.0	10.2	0.1	**0.4**	-0.2	0.1		
			3	28.9	28.9	30.0	**29.3**	17.5	17.6	17.1	**17.4**	11.9	10.2	0.3	**0.4**	-0.1	0.0	0.1	**0.3**
	4	suc	1	27.4	27.4	27.6	**27.5**	16.4	16.5	16.4	**16.4**	11.0	9.4	0.3	**1.3**	-1.0	1.0		
			2	32.0	31.1	32.6	**31.9**	23.4	23.6	23.6	**23.5**	8.4	9.4	2.0	**1.3**	0.7	0.5		
			3	24.0	24.4	24.4	**24.3**	15.4	15.3	15.8	**15.5**	8.8	9.4	1.5	**1.3**	0.2	0.1	1.6	**0.9**
		glc	1	24.7	24.8	24.7	**24.7**	16.1	16.0	16.3	**16.1**	8.6	9.4	1.7	**1.0**	0.7	0.5		
			2	25.7	25.6	25.5	**25.6**	15.8	15.6	16.0	**15.8**	9.8	9.4	0.8	**1.0**	-0.3	0.1		
			3	25.8	25.8	26.0	**25.9**	15.9	15.7	15.9	**15.8**	10.0	9.4	0.6	**1.0**	-0.4	0.2	0.7	**0.6**
		fru	1	29.9	29.6	30.5	**30.0**	20.3	20.4	20.9	**20.5**	9.5	9.4	0.9	**0.9**	0.0	0.0		
			2	28.5	28.4	28.1	**28.3**	19.0	18.5	18.8	**18.8**	9.6	9.4	0.9	**0.9**	-0.1	0.0		
			3	28.0	28.0	28.0	**28.0**	18.5	18.7	18.6	**18.6**	9.4	9.4	1.0	**0.9**	0.1	0.0	0.0	**0.1**
	7	suc	1	31.3	31.4	31.4	**31.4**	19.7	19.9	19.8	**19.8**	11.6	11.3	0.9	**1.0**	-0.2	0.0		

		3	32.6	32.4	33.4	**32.8**	21.5	21.2	21.6	**21.4**	11.4	11.3	1.0	**1.0**	0.0	0.0	0.1	**0.2**
	glc	1	31.0	31.2	30.8	**31.0**	20.8	20.6	20.7	**20.7**	10.3	11.3	2.0	**1.8**	0.3	0.1		
		2	29.7	29.6	29.6	**29.6**	19.5	19.4	19.4	**19.4**	10.2	11.3	2.2	**1.8**	0.4	0.2		
		3	30.8	30.0	31.1	**30.6**	19.4	19.5	19.4	**19.4**	11.2	11.3	1.1	**1.8**	-0.7	0.5	0.7	**0.6**
	fru	1	29.8	29.9	29.9	**29.9**	19.1	19.1	19.1	**19.1**	10.8	11.3	1.5	**1.3**	0.2	0.0		
		2	29.0	29.5	29.7	**29.4**	18.2	18.3	18.4	**18.3**	11.1	11.3	1.2	**1.3**	-0.1	0.0		
		3	29.9	30.3	30.1	**30.1**	19.1	19.1	19.1	**19.1**	11.0	11.3	1.3	**1.3**	0.0	0.0	0.0	**0.2**
14	suc	1	29.5	29.8	29.1	**29.5**	20.3	20.2	19.8	**20.1**	9.4	9.5	1.1	**1.4**	-0.3	0.1		
		2	28.7	28.8	29.1	**28.9**	17.5	17.4	18.5	**17.8**	11.1	9.5	0.3	**1.4**	-1.1	1.1		
		3	28.6	29.0	29.1	**28.9**	20.5	21.2	21.0	**20.9**	8.0	9.5	2.8	**1.4**	1.4	1.9	3.2	**1.3**
	glc	1	29.1	29.1	29.1	**29.1**	21.1	21.6	21.4	**21.4**	7.7	9.5	3.4	**7.1**	-3.7	14.0		
		2	26.1	26.1	26.1	**26.1**	20.0	20.0	20.0	**20.0**	6.1	9.5	10.4	**7.1**	3.3	10.9		
		3	26.3	26.4	26.4	**26.4**	19.7	20.0	19.7	**19.8**	6.6	9.5	7.5	**7.1**	0.4	0.2	25.1	**3.5**
	fru	1	28.4	28.6	29.3	**28.8**	19.5	20.0	20.2	**19.9**	8.9	9.5	1.5	**0.7**	0.8	0.6		
		2	29.2	29.3	29.6	**29.4**	18.7	18.7	19.3	**18.9**	10.5	9.5	0.5	**0.7**	-0.2	0.1		

Table A3. Real-time PCR data and calculation of fold change and standard deviation for sucrose, glucose and fructose treatments at days 1, 2, 3, 4, 7, and 14 relative to pre-treatment at day 0.

Gene	Day	Treatment	Sample	CT 1	CT 2	CT 3	Avg CT	18S CT 1	18S CT 2	18S CT3	Avg 18S CT	ΔCT	Avg D0 ΔCT	Fold Change	Avg Fold Change	Deviation	Deviation^2	ΣDeviation^2	SD
ADS	0	none	1	32.6	32.3	32.5	**32.5**	19.1	18.2	18.1	**18.5**	14.0	13.6	0.8	**1.0**	-0.3	0.1		
			2	32.2	32.0	32.2	**32.1**	18.9	18.9	18.8	**18.9**	13.3	13.6	1.3	**1.0**	0.3	0.1		
			3	29.0	28.9	29.7	**29.2**	15.9	15.5	15.4	**15.6**	13.6	13.6	1.0	**1.0**	0.0	0.0	0.1	**0.3**
	1	suc	1	33.2	32.1	33.2	**32.8**	19.3	19.1	19.1	**19.2**	13.7	13.6	1.0	**1.2**	-0.2	0.0		
			2	33.1	33.5	33.2	**33.3**	19.7	19.5	19.6	**19.6**	13.7	13.6	1.0	**1.2**	-0.2	0.0		
			3	30.2	30.4	31.1	**30.6**	17.6	17.6	17.7	**17.6**	12.9	13.6	1.6	**1.2**	0.4	0.2	0.3	**0.4**
		glc	1	33.2	32.7	32.2	**32.7**	17.0	17.1	17.0	**17.0**	15.7	13.6	0.2	**0.5**	-0.2	0.0		
			2	33.4	33.5	33.1	**33.3**	18.5	18.8	18.7	**18.7**	14.7	13.6	0.5	**0.5**	0.0	0.0		
			3	33.5	33.1	32.9	**33.2**	18.8	19.0	18.9	**18.9**	14.3	13.6	0.6	**0.5**	0.2	0.0	0.1	**0.2**
		fru	1	30.6	31.0	30.9	**30.8**	16.6	16.6	16.7	**16.6**	14.2	13.6	0.7	**0.6**	0.1	0.0		
			2	33.1	33.0	32.9	**33.0**	18.2	18.4	18.6	**18.4**	14.6	13.6	0.5	**0.6**	-0.1	0.0		
			3	29.8	29.9	30.2	**30.0**	16.0	15.9	15.4	**15.8**	14.2	13.6	0.7	**0.6**	0.1	0.0	0.0	**0.1**
	2	suc	1	29.7	29.6	29.9	**29.7**	18.3	18.4	18.7	**18.5**	11.3	13.6	5.1	**6.1**	-1.0	1.0		
			2	29.2	29.6	29.7	**29.5**	18.8	18.7	18.7	**18.7**	10.8	13.6	7.2	**6.1**	1.1	1.2		
			3	31.1	29.9	30.7	**30.6**	19.7	19.4	19.5	**19.5**	11.0	13.6	6.0	**6.1**	-0.1	0.0	2.3	**1.1**
		glc	1	28.1	28.2	28.4	**28.2**	17.1	17.2	17.0	**17.1**	11.1	13.6	5.6	**6.3**	-0.7	0.4		
			2	26.9	26.9	26.9	**26.9**	16.4	16.0	16.2	**16.2**	10.7	13.6	7.6	**6.3**	1.3	1.7		
			3	29.8	30.0	29.9	**29.9**	18.7	18.8	18.8	**18.8**	11.1	13.6	5.6	**6.3**	-0.7	0.4	2.6	**1.1**
		fru	1	28.7	29.3	29.3	**29.1**	16.7	16.7	16.7	**16.7**	12.4	13.6	2.3	**1.9**	0.4	0.2		
			2	29.6	29.9	29.7	**29.7**	17.1	16.9	17.1	**17.0**	12.7	13.6	1.9	**1.9**	0.0	0.0		
			3	29.0	29.3	29.3	**29.2**	16.0	16.0	16.5	**16.2**	13.0	13.6	1.5	**1.9**	-0.4	0.2	0.3	**0.4**
	3	suc	1	33.4	33.3	33.4	**33.4**	22.7	23.0	23.1	**22.9**	10.4	13.6	9.1	**8.6**	0.5	0.3		
			2	24.3	24.3	24.4	**24.3**	13.8	13.6	13.7	**13.7**	10.6	13.6	7.9	**8.6**	-0.6	0.4		
			3	26.2	26.0	26.1	**26.1**	15.7	15.5	15.6	**15.6**	10.5	13.6	8.7	**8.6**	0.1	0.0	0.7	**0.6**
		glc	1	31.5	30.9	31.2	**31.2**	21.0	21.1	21.5	**21.2**	10.0	13.6	12.3	**11.8**	0.5	0.2		
			2	34.9	34.5	34.6	**34.7**	24.8	24.8	24.6	**24.7**	9.9	13.6	12.9	**11.8**	1.1	1.2		
			3	33.5	33.5	33.4	**33.5**	23.3	23.2	23.1	**23.2**	10.3	13.6	10.2	**11.8**	-1.6	2.5	3.9	**1.4**
		fru	1	33.3	33.3	33.3	**33.3**	20.7	20.8	20.9	**20.8**	12.5	13.6	2.2	**2.2**	0.0	0.0		
			2	34.3	34.5	34.4	**34.4**	21.6	21.8	21.5	**21.6**	12.8	13.6	1.8	**2.2**	-0.4	0.1		
			3	34.5	34.0	34.1	**34.2**	21.8	22.0	21.9	**21.9**	12.3	13.6	2.5	**2.2**	0.3	0.1	0.2	**0.3**
	4	suc	1	31.6	31.6	31.6	**31.6**	21.5	21.5	21.4	**21.5**	10.1	13.6	11.2	**12.2**	-1.0	1.0		
			2	30.9	30.8	30.8	**30.8**	20.6	20.8	20.6	**20.7**	10.2	13.6	11.0	**12.2**	-1.3	1.6		
			3	24.9	24.8	24.8	**24.8**	14.8	15.1	15.3	**15.1**	9.8	13.6	14.5	**12.2**	2.3	5.1	7.6	**2.0**
		glc	1	26.4	26.4	26.4	**26.4**	16.7	16.8	16.8	**16.8**	9.6	13.6	15.9	**20.3**	-4.4	19.5		
			2	27.5	27.5	27.5	**27.5**	18.3	18.6	18.5	**18.5**	9.0	13.6	24.1	**20.3**	3.8	14.2		
			3	28.6	28.4	28.4	**28.5**	19.0	19.4	19.3	**19.2**	9.2	13.6	21.0	**20.3**	0.7	0.4	34.2	**4.1**
		fru	1	30.4	30.4	30.3	**30.4**	17.1	17.4	17.6	**17.4**	13.0	13.6	1.5	**2.2**	-0.7	0.5		
			2	31.1	31.1	31.1	**31.1**	19.0	18.8	18.7	**18.8**	12.3	13.6	2.6	**2.2**	0.3	0.1		
			3	33.4	33.4	33.3	**33.4**	20.9	21.2	21.3	**21.1**	12.2	13.6	2.6	**2.2**	0.4	0.1	0.7	**0.6**
	7	suc	1	26.0	26.7	25.8	**26.2**	15.5	15.8	16.1	**15.8**	10.4	13.6	9.6	**10.5**	-1.0	0.9		
			2	24.0	24.5	24.7	**24.4**	14.5	14.5	14.6	**14.5**	9.9	13.6	13.5	**10.5**	3.0	8.9		
			3	28.0	28.0	27.9	**28.0**	17.7	16.7	17.9	**17.4**	10.5	13.6	8.5	**10.5**	-2.0	4.1	13.9	**2.6**
		glc	1	23.7	23.7	23.8	**23.7**	15.0	15.8	15.9	**15.6**	8.2	13.6	43.9	**37.0**	6.9	47.2		
			2	24.0	24.2	24.3	**24.2**	15.9	15.1	15.2	**15.4**	8.8	13.6	29.0	**37.0**	-8.1	65.0		
			3	26.0	25.9	25.9	**25.9**	17.7	17.3	17.7	**17.6**	8.4	13.6	38.2	**37.0**	1.2	1.4	113.6	**7.5**
		fru	1	26.5	26.4	26.6	**26.5**	15.6	15.2	14.8	**15.2**	11.3	13.6	5.0	**5.5**	-0.5	0.2		
			2	26.1	26.0	25.9	**26.0**	14.7	15.4	15.6	**15.2**	10.8	13.6	7.2	**5.5**	1.8	3.1		
			3	26.8	26.9	26.9	**26.9**	15.5	15.6	14.8	**15.3**	11.6	13.6	4.2	**5.5**	-1.3	1.7	5.1	**1.6**
	14	suc	1	23.9	23.9	24.5	**24.1**	14.3	14.2	14.1	**14.2**	9.9	13.6	13.2	**13.8**	-0.6	0.4		
			2	25.2	24.9	25.6	**25.2**	14.7	15.5	14.8	**15.0**	10.2	13.6	10.5	**13.8**	-3.4	11.3		

			3	24.5	24.6	24.9	**24.7**	14.9	15.0	15.7	**15.2**	9.5	13.6	17.8	**13.8**	4.0	15.9	27.6	**3.7**
		glc	1	23.2	22.8	23.1	**23.0**	14.7	14.6	14.7	**14.7**	8.4	13.6	38.2	**42.5**	-4.3	18.5		
			2	21.6	21.6	21.6	**21.6**	13.8	13.9	13.6	**13.8**	7.8	13.6	55.3	**42.5**	12.8	163.3		
			3	22.3	22.4	22.3	**22.3**	13.7	13.8	13.9	**13.8**	8.5	13.6	34.0	**42.5**	-8.5	71.8	253.7	**11.3**
		fru	1	24.2	24.6	24.9	**24.6**	14.8	14.8	15.1	**14.9**	9.7	13.6	15.5	**10.6**	4.9	24.5		
			2	25.1	25.0	24.8	**25.0**	14.7	14.5	14.4	**14.5**	10.4	13.6	9.1	**10.6**	-1.4	2.1		
			3	25.3	25.5	25.5	**25.4**	14.6	14.7	14.6	**14.6**	10.8	13.6	7.1	**10.6**	-3.5	12.2	38.8	**4.4**

Gene	Day	Treatment	Sample	CT 1	CT 2	CT 3	Avg CT	18S CT 1	18S CT 2	18S CT3	Avg 18S CT	ΔCT	Avg D0 ΔCT	Fold Change	Avg Fold Change	Deviation	Deviation²	ΣDeviation²	SD
CYP	0	none	1	32.7	31.9	32.0	**32.2**	16.4	16.4	16.4	**16.4**	15.8	14.8	0.5	**1.1**	-0.7	0.4		
			2	33.0	32.9	32.5	**32.8**	18.4	18.3	18.1	**18.3**	14.5	14.8	1.2	**1.1**	0.0	0.0		
			3	30.9	30.1	30.1	**30.4**	16.4	16.3	16.6	**16.4**	13.9	14.8	1.8	**1.1**	0.6	0.4	0.8	**0.6**
	1	suc	1	32.3	33.1	32.7	**32.7**	19.3	19.1	19.4	**19.3**	13.4	14.8	2.5	**2.7**	-0.2	0.1		
			2	33.3	33.4	33.7	**33.5**	19.6	19.8	19.8	**19.7**	13.7	14.8	2.0	**2.7**	-0.7	0.5		
			3	30.6	30.5	30.4	**30.5**	17.6	17.6	17.7	**17.6**	12.9	14.8	3.7	**2.7**	1.0	0.9	1.5	**0.9**
		glc	1	31.9	32.2	32.0	**32.0**	17.8	17.8	17.8	**17.8**	14.2	14.8	1.4	**1.4**	0.1	0.0		
			2	33.2	33.1	32.9	**33.1**	19.0	19.0	18.9	**19.0**	14.1	14.8	1.6	**1.4**	0.2	0.0		
			3	33.4	33.5	33.7	**33.5**	18.8	19.0	18.9	**18.9**	14.6	14.8	1.1	**1.4**	-0.3	0.1	0.1	**0.3**
		fru	1	30.7	30.2	30.3	**30.4**	16.7	16.7	16.7	**16.7**	13.7	14.8	2.1	**1.3**	0.8	0.6		
			2	31.2	31.0	32.5	**31.6**	17.2	17.3	17.2	**17.2**	14.3	14.8	1.3	**1.3**	0.0	0.0		
			3	31.7	31.0	31.4	**31.4**	16.0	15.9	15.4	**15.8**	15.6	14.8	0.6	**1.3**	-0.8	0.6	1.2	**0.8**
	2	suc	1	30.4	30.4	30.3	**30.4**	18.6	18.4	18.6	**18.5**	11.8	14.8	7.6	**8.6**	-1.0	1.0		
			2	29.4	29.3	29.4	**29.4**	17.7	17.6	17.5	**17.6**	11.8	14.8	7.9	**8.6**	-0.6	0.4		
			3	27.9	28.0	27.8	**27.9**	16.6	16.5	16.4	**16.5**	11.4	14.8	10.2	**8.6**	1.7	2.7	4.2	**1.4**
		glc	1	26.7	27.1	26.7	**26.8**	16.5	16.6	16.4	**16.5**	10.3	14.8	21.4	**19.7**	1.7	2.9		
			2	25.9	25.7	25.8	**25.8**	15.6	15.4	15.7	**15.6**	10.2	14.8	23.0	**19.7**	3.2	10.5		
			3	28.6	28.7	28.9	**28.7**	17.7	18.1	17.8	**17.9**	10.9	14.8	14.8	**19.7**	-4.9	24.3	37.6	**4.3**
		fru	1	26.9	26.7	26.8	**26.8**	15.6	15.6	15.5	**15.6**	11.2	14.8	11.5	**9.3**	2.2	4.6		
			2	29.6	29.2	29.4	**29.4**	17.7	17.5	17.4	**17.5**	11.9	14.8	7.4	**9.3**	-1.9	3.7		
			3	28.6	28.2	28.3	**28.4**	16.8	16.7	16.9	**16.8**	11.6	14.8	9.1	**9.3**	-0.2	0.0	8.4	**2.0**
	3	suc	1	29.6	29.4	29.3	**29.4**	17.2	18.0	17.4	**17.5**	11.9	14.8	7.2	**9.8**	-2.6	6.7		
			2	24.2	24.2	24.6	**24.3**	13.2	13.0	13.2	**13.1**	11.2	14.8	11.8	**9.8**	1.9	3.7		
			3	24.3	24.5	24.6	**24.5**	13.1	13.1	13.1	**13.1**	11.4	14.8	10.5	**9.8**	0.7	0.4	10.9	**2.3**
		glc	1	28.2	28.2	28.3	**28.2**	15.9	15.8	16.0	**15.9**	12.3	14.8	5.4	**4.2**	1.1	1.3		
			2	31.2	31.4	31.4	**31.3**	18.7	18.7	18.5	**18.6**	12.7	14.8	4.2	**4.2**	-0.1	0.0		
			3	29.9	29.8	30.0	**29.9**	16.8	16.8	16.8	**16.8**	13.1	14.8	3.2	**4.2**	-1.1	1.1	2.4	**1.1**
		fru	1	29.0	29.3	29.5	**29.3**	16.3	16.8	16.0	**16.4**	12.9	14.8	3.6	**3.7**	-0.1	0.0		
			2	31.4	30.3	31.9	**31.2**	17.6	17.9	17.9	**17.8**	13.4	14.8	2.6	**3.7**	-1.2	1.4		
			3	30.8	30.9	31.2	**31.0**	18.5	18.5	18.6	**18.5**	12.4	14.8	5.0	**3.7**	1.3	1.6	3.0	**1.2**
	4	suc	1	29.1	29.4	28.8	**29.1**	16.8	17.3	17.2	**17.1**	12.0	14.8	6.8	**9.3**	-2.5	6.3		
			2	29.5	29.7	29.8	**29.7**	17.9	18.0	18.2	**18.0**	11.6	14.8	8.7	**9.3**	-0.6	0.3		
			3	25.6	25.8	25.7	**25.7**	14.5	14.5	14.7	**14.6**	11.1	14.8	12.3	**9.3**	3.1	9.3	15.9	**2.8**
		glc	1	26.1	26.0	26.6	**26.2**	14.7	14.5	14.8	**14.7**	11.6	14.8	9.1	**9.9**	-0.8	0.6		
			2	26.2	26.3	26.4	**26.3**	15.4	15.1	15.3	**15.3**	11.0	14.8	13.2	**9.9**	3.3	10.8		
			3	27.6	27.2	27.6	**27.5**	15.5	15.6	15.7	**15.6**	11.9	14.8	7.4	**9.9**	-2.5	6.3	17.7	**3.0**
		fru	1	28.9	29.3	29.6	**29.3**	16.0	16.1	16.4	**16.2**	13.1	14.8	3.2	**3.9**	-0.7	0.5		
			2	28.3	27.9	27.9	**28.0**	15.9	15.8	15.7	**15.8**	12.2	14.8	5.7	**3.9**	1.9	3.5		
			3	33.4	33.4	33.2	**33.3**	20.1	20.1	19.9	**20.0**	13.3	14.8	2.7	**3.9**	-1.1	1.3	5.3	**1.6**
	7	suc	1	28.5	28.3	28.2	**28.3**	15.5	15.8	16.1	**15.8**	12.5	14.8	4.7	**7.8**	-3.1	9.7		
			2	26.0	25.8	26.3	**26.0**	14.5	14.5	14.6	**14.5**	11.5	14.8	9.6	**7.8**	1.8	3.1		
			3	29.0	28.9	29.1	**29.0**	17.7	16.7	17.9	**17.4**	11.6	14.8	9.1	**7.8**	1.3	1.8	14.6	**2.7**
		glc	1	25.7	25.4	25.3	**25.5**	15.0	15.8	15.9	**15.6**	9.9	14.8	29.0	**25.0**	3.9	15.4		
			2	25.9	26.0	25.8	**25.9**	15.9	15.1	15.2	**15.4**	10.5	14.8	19.1	**25.0**	-5.9	35.1		
			3	27.5	27.4	27.8	**27.6**	17.7	17.3	17.7	**17.6**	10.0	14.8	27.0	**25.0**	2.0	4.0	54.5	**5.2**

		fru	1	27.3	27.6	27.3	**27.4**	15.6	15.2	14.8	**15.2**	12.2	14.8	5.9	**5.9**	-0.1	0.0		
			2	28.9	28.6	28.7	**28.7**	14.7	15.4	15.6	**15.2**	13.5	14.8	2.4	**5.9**	-3.6	12.6		
			3	26.8	26.6	27.0	**26.8**	15.5	15.6	14.8	**15.3**	11.5	14.8	9.6	**5.9**	3.6	13.0	25.7	**3.6**
	14	suc	1	25.5	25.5	25.8	**25.6**	14.3	14.2	14.1	**14.2**	11.4	14.8	10.2	**9.5**	0.7	0.5		
			2	26.7	26.8	27.1	**26.9**	14.7	15.5	14.8	**15.0**	11.9	14.8	7.4	**9.5**	-2.1	4.5		
			3	26.3	26.3	26.9	**26.5**	14.9	15.0	15.7	**15.2**	11.3	14.8	11.0	**9.5**	1.4	2.1	7.1	**1.9**
		glc	1	25.9	25.7	26.2	**25.9**	14.7	14.6	14.7	**14.7**	11.3	14.8	11.2	**14.6**	-3.4	11.3		
			2	24.0	24.0	24.0	**24.0**	13.8	13.9	13.6	**13.8**	10.2	14.8	23.0	**14.6**	8.4	70.4		
			3	25.3	25.1	25.5	**25.3**	13.7	13.8	13.9	**13.8**	11.5	14.8	9.6	**14.6**	-5.0	25.4	107.1	**7.3**
		fru	1	26.5	26.5	26.4	**26.5**	14.8	14.8	15.1	**14.9**	11.6	14.8	9.1	**7.1**	2.0	4.1		
			2	26.4	26.2	26.5	**26.4**	14.7	14.5	14.4	**14.5**	11.8	14.8	7.6	**7.1**	0.5	0.2		
			3	27.7	27.1	26.8	**27.2**	14.6	14.7	14.6	**14.6**	12.6	14.8	4.6	**7.1**	-2.5	6.4	10.8	**2.3**
Gene	**Day**	**Treatment**	**Sample**	**CT 1**	**CT 2**	**CT 3**	**Avg CT**	**18S CT 1**	**18S CT 2**	**18S CT3**	**Avg 18S CT**	**ΔCT**	**Avg D0 ΔCT**	**Fold Change**	**Avg Fold Change**	**Deviation**	**Deviation^2**	**ΣDeviation^2**	**SD**
FPS	0	none	1	31.5	31.7	31.8	**31.7**	19.8	19.7	20.0	**19.8**	11.8	11.0	0.6	**1.1**	-0.5	0.3		
			2	32.4	32.6	32.8	**32.6**	21.4	21.9	21.1	**21.5**	11.1	11.0	0.9	**1.1**	-0.2	0.0		
			3	30.6	30.4	30.1	**30.4**	20.1	20.3	20.2	**20.2**	10.2	11.0	1.8	**1.1**	0.7	0.5	0.8	**0.6**
	1	suc	1	32.9	32.3	32.2	**32.5**	22.1	21.9	22.1	**22.0**	10.4	11.0	1.5	**1.5**	0.0	0.0		
			2	33.4	33.5	33.3	**33.4**	23.3	23.5	23.2	**23.3**	10.1	11.0	2.0	**1.5**	0.4	0.2		
			3	31.4	31.9	31.9	**31.7**	20.8	20.9	20.9	**20.9**	10.9	11.0	1.1	**1.5**	-0.4	0.2	0.4	**0.4**
		glc	1	32.5	32.7	33.0	**32.7**	25.0	25.6	24.8	**25.1**	7.6	11.0	10.9	**12.4**	-1.5	2.3		
			2	32.9	33.4	33.5	**33.3**	25.5	25.1	25.8	**25.5**	7.8	11.0	9.5	**12.4**	-2.9	8.6		
			3	30.8	31.0	31.7	**31.2**	24.0	24.1	24.5	**24.2**	7.0	11.0	16.9	**12.4**	4.5	20.0	31.0	**3.9**
		fru	1	30.4	30.3	30.1	**30.3**	20.4	20.0	20.4	**20.3**	10.0	11.0	2.1	**3.1**	-1.0	1.0		
			2	33.0	32.4	32.2	**32.5**	23.4	23.4	23.7	**23.5**	9.0	11.0	4.0	**3.1**	1.0	0.9		
			3	32.1	32.2	32.0	**32.1**	22.6	22.8	22.7	**22.7**	9.4	11.0	3.1	**3.1**	0.1	0.0	1.9	**1.0**
	2	suc	1	31.5	31.6	31.5	**31.5**	21.7	21.0	21.6	**21.4**	10.1	11.0	1.9	**2.7**	-0.8	0.7		
			2	30.2	30.4	30.4	**30.3**	21.1	20.9	21.6	**21.2**	9.1	11.0	3.8	**2.7**	1.0	1.0		
			3	32.1	32.2	32.6	**32.3**	22.2	23.0	22.6	**22.6**	9.7	11.0	2.5	**2.7**	-0.2	0.0	1.7	**0.9**
		glc	1	28.8	29.0	29.2	**29.0**	21.1	21.4	20.9	**21.1**	7.9	11.0	9.0	**9.8**	-0.7	0.5		
			2	27.2	26.9	27.1	**27.1**	19.5	19.6	19.6	**19.6**	7.5	11.0	11.7	**9.8**	1.9	3.5		
			3	32.3	32.2	32.4	**32.3**	24.4	24.2	24.5	**24.4**	7.9	11.0	8.6	**9.8**	-1.1	1.3	5.4	**1.6**
		fru	1	27.7	27.6	27.8	**27.7**	18.6	18.4	18.7	**18.6**	9.1	11.0	3.8	**2.4**	1.4	2.0		
			2	29.5	29.7	30.1	**29.8**	19.7	19.7	19.7	**19.7**	10.1	11.0	2.0	**2.4**	-0.4	0.2		
			3	29.6	29.5	29.6	**29.6**	18.9	18.9	19.1	**19.0**	10.6	11.0	1.4	**2.4**	-1.0	1.0	3.1	**1.2**
	3	suc	1	21.6	21.0	21.4	**21.3**	12.3	12.5	12.4	**12.4**	8.9	11.0	4.3	**5.4**	-1.1	1.1		
			2	20.7	20.6	20.9	**20.7**	11.5	11.7	11.8	**11.7**	9.1	11.0	3.9	**5.4**	-1.4	2.1		
			3	18.6	18.4	18.8	**18.6**	10.7	10.4	10.5	**10.5**	8.1	11.0	7.9	**5.4**	2.5	6.2	9.4	**2.2**
		glc	1	20.2	20.0	20.0	**20.1**	11.4	11.6	11.8	**11.6**	8.5	11.0	6.0	**6.3**	-0.3	0.1		
			2	21.8	21.9	21.0	**21.6**	13.0	12.8	12.5	**12.8**	8.8	11.0	4.7	**6.3**	-1.6	2.5		
			3	18.2	19.0	18.6	**18.6**	10.7	10.7	10.4	**10.6**	8.0	11.0	8.3	**6.3**	1.9	3.7	6.3	**1.8**
		fru	1	19.0	18.5	18.6	**18.7**	10.1	10.1	10.0	**10.1**	8.6	11.0	5.3	**3.9**	1.4	2.1		
			2	20.3	20.4	20.6	**20.4**	10.8	11.0	11.2	**11.0**	9.4	11.0	3.1	**3.9**	-0.8	0.7		
			3	21.0	20.8	20.9	**20.9**	11.8	11.5	11.4	**11.6**	9.3	11.0	3.3	**3.9**	-0.6	0.4	3.1	**1.2**
	4	suc	1	18.7	18.8	18.7	**18.7**	9.7	9.6	9.8	**9.7**	9.0	11.0	4.0	**5.3**	-1.2	1.5		
			2	19.0	19.2	19.4	**19.2**	10.9	10.7	11.0	**10.9**	8.3	11.0	6.5	**5.3**	1.3	1.7		
			3	17.3	17.6	17.9	**17.6**	8.7	9.0	9.1	**8.9**	8.7	11.0	5.2	**5.3**	-0.1	0.0	3.2	**1.3**
		glc	1	18.6	18.3	18.4	**18.4**	10.1	9.8	10.0	**10.0**	8.5	11.0	6.0	**3.9**	2.0	4.1		
			2	19.3	19.5	19.1	**19.3**	10.1	10.0	9.9	**10.0**	9.3	11.0	3.4	**3.9**	-0.6	0.3		
			3	20.0	19.9	19.8	**19.9**	10.1	10.1	10.3	**10.2**	9.7	11.0	2.5	**3.9**	-1.5	2.1	6.6	**1.8**
		fru	1	22.4	20.9	21.8	**21.7**	12.5	12.4	13.0	**12.6**	9.1	11.0	3.9	**4.2**	-0.3	0.1		
			2	18.9	18.6	19.2	**18.9**	10.0	10.2	10.3	**10.2**	8.7	11.0	5.0	**4.2**	0.8	0.6		
			3	21.1	21.1	21.5	**21.2**	12.0	12.1	12.1	**12.1**	9.2	11.0	3.7	**4.2**	-0.5	0.3	0.9	**0.7**
	7	suc	1	26.6	26.4	26.4	**26.5**	16.7	16.8	16.7	**16.7**	9.7	11.0	2.5	**3.5**	-1.0	1.1		

			2	24.7	24.8	24.8	24.8	16.1	16.1	16.0	16.1	8.7	11.0	5.1	3.5	1.6	2.4		
			3	28.0	27.9	27.9	27.9	18.5	18.3	18.6	18.5	9.5	11.0	3.0	3.5	-0.5	0.3	3.8	1.4
		glc	1	23.6	23.7	24.0	23.8	14.9	15.1	15.2	15.1	8.7	11.0	5.1	5.6	-0.6	0.3		
			2	22.8	22.6	22.7	22.7	14.0	13.8	13.8	13.9	8.8	11.0	4.6	5.6	-1.0	1.0		
			3	28.1	28.0	28.1	28.1	19.9	19.9	19.8	19.9	8.2	11.0	7.2	5.6	1.6	2.4	3.7	1.4
		fru	1	23.6	23.6	23.5	23.6	14.1	14.2	14.1	14.1	9.4	11.0	3.1	3.1	0.0	0.0		
			2	23.7	23.8	24.3	23.9	14.2	14.3	14.8	14.4	9.5	11.0	2.9	3.1	-0.1	0.0		
			3	23.9	23.7	23.8	23.8	14.5	14.4	14.4	14.4	9.4	11.0	3.2	3.1	0.1	0.0	0.0	0.1
	14	suc	1	24.0	24.2	24.0	24.1	15.7	15.7	15.6	15.7	8.4	11.0	6.3	4.9	1.4	1.9		
			2	22.9	23.1	23.1	23.0	14.6	14.6	14.6	14.6	8.4	11.0	6.1	4.9	1.2	1.5		
			3	24.7	24.8	25.1	24.9	14.9	14.9	15.2	15.0	9.9	11.0	2.3	4.9	-2.6	6.8	10.2	2.3
		glc	1	22.5	22.2	22.2	22.3	14.2	14.6	14.2	14.3	8.0	11.0	8.4	6.1	2.3	5.4		
			2	23.1	23.0	23.0	23.0	14.9	14.9	14.8	14.9	8.2	11.0	7.4	6.1	1.2	1.5		
			3	22.5	22.4	22.7	22.5	12.8	12.8	12.9	12.8	9.7	11.0	2.5	6.1	-3.6	12.8	19.7	3.1
		fru	1	21.8	21.9	22.2	22.0	14.0	14.1	14.3	14.1	7.8	11.0	9.3	6.4	2.8	8.0		
			2	22.9	23.0	22.8	22.9	14.7	14.6	14.6	14.6	8.3	11.0	6.9	6.4	0.4	0.2		
			3	24.5	24.6	24.7	24.6	15.1	15.3	15.3	15.2	9.4	11.0	3.2	6.4	-3.2	10.5	18.6	3.1
Gene	**Day**	**Treatment**	**Sample**	**CT 1**	**CT 2**	**CT 3**	**Avg CT**	**18S CT 1**	**18S CT 2**	**18S CT3**	**Avg 18S CT**	**ΔCT**	**Avg D0 ΔCT**	**Fold Change**	**Avg Fold Change**	**Deviation**	**Deviation^2**	**ΣDeviation^2**	**SD**
HMGR	0	none	1	29.2	29.1	29.1	29.1	19.8	19.7	20.0	19.8	9.3	8.4	0.5	1.1	-0.6	0.3		
			2	29.2	29.6	29.6	29.5	21.4	21.9	21.1	21.5	8.0	8.4	1.3	1.1	0.2	0.0		
			3	28.2	27.8	28.0	28.0	20.1	20.3	20.2	20.2	7.8	8.4	1.5	1.1	0.4	0.1	0.5	0.5
	1	suc	1	30.9	31.4	31.6	31.3	22.1	21.9	22.1	22.0	9.3	8.4	0.5	1.0	-0.4	0.2		
			2	31.4	31.1	31.1	31.2	23.3	23.5	23.2	23.3	7.9	8.4	1.4	1.0	0.5	0.2		
			3	28.8	29.7	29.5	29.3	20.8	20.9	20.9	20.9	8.5	8.4	0.9	1.0	0.0	0.0	0.4	0.4
		glc	1	31.6	31.6	31.1	31.4	25.0	25.6	24.8	25.1	6.3	8.4	4.2	3.2	1.0	0.9		
			2	32.2	33.3	32.0	32.5	25.5	25.1	25.8	25.5	7.0	8.4	2.5	3.2	-0.7	0.5		
			3	30.7	31.1	31.2	31.0	24.0	24.1	24.5	24.2	6.8	8.4	3.0	3.2	-0.3	0.1	1.5	0.9
		fru	1	27.7	28.0	27.7	27.8	20.4	20.0	20.4	20.3	7.5	8.4	1.8	2.2	-0.4	0.2		
			2	30.9	30.5	30.7	30.7	23.4	23.4	23.7	23.5	7.2	8.4	2.2	2.2	0.0	0.0		
			3	29.8	29.7	29.6	29.7	22.6	22.8	22.7	22.7	7.0	8.4	2.6	2.2	0.4	0.1	0.3	0.4
	2	suc	1	29.3	30.5	29.7	29.8	21.7	21.0	21.6	21.4	8.4	8.4	1.0	1.6	-0.6	0.3		
			2	28.7	29.2	28.8	28.9	21.1	20.9	21.6	21.2	7.7	8.4	1.6	1.6	0.0	0.0		
			3	29.8	30.1	29.8	29.9	22.2	23.0	22.6	22.6	7.3	8.4	2.1	1.6	0.5	0.3	0.6	0.6
		glc	1	30.0	29.9	29.5	29.8	21.1	21.4	20.9	21.1	8.7	8.4	0.8	0.9	-0.1	0.0		
			2	29.0	28.9	29.2	29.0	19.5	19.6	19.6	19.6	9.5	8.4	0.5	0.9	-0.5	0.2		
			3	32.0	32.4	32.0	32.1	24.4	24.2	24.5	24.4	7.8	8.4	1.5	0.9	0.6	0.3	0.6	0.5
		fru	1	28.1	28.1	28.5	28.2	18.6	18.4	18.7	18.6	9.7	8.4	0.4	0.5	-0.1	0.0		
			2	28.8	28.5	28.8	28.7	19.7	19.7	19.7	19.7	9.0	8.4	0.6	0.5	0.2	0.0		
			3	28.6	28.8	29.1	28.8	18.9	18.9	19.1	19.0	9.9	8.4	0.4	0.5	-0.1	0.0	0.0	0.2
	3	suc	1	21.2	20.5	21.0	20.9	12.3	12.5	12.4	12.4	8.5	8.4	0.9	1.5	-0.6	0.4		
			2	19.8	19.9	19.7	19.8	11.5	11.7	11.8	11.7	8.1	8.4	1.2	1.5	-0.3	0.1		
			3	17.2	18.0	17.6	17.6	10.7	10.4	10.5	10.5	7.1	8.4	2.5	1.5	0.9	0.9	1.4	0.8
		glc	1	19.8	19.8	19.5	19.7	11.4	11.6	11.8	11.6	8.1	8.4	1.2	1.4	-0.2	0.0		
			2	20.3	20.4	20.2	20.3	13.0	12.8	12.5	12.8	7.5	8.4	1.8	1.4	0.4	0.2		
			3	18.4	19.1	18.7	18.7	10.7	10.7	10.4	10.6	8.1	8.4	1.2	1.4	-0.2	0.0	0.2	0.3
		fru	1	19.5	19.5	19.6	19.5	10.1	10.1	10.0	10.1	9.5	8.4	0.5	0.8	-0.3	0.1		
			2	19.3	19.2	19.0	19.2	10.8	11.0	11.2	11.0	8.2	8.4	1.1	0.8	0.4	0.1		
			3	20.7	20.3	20.0	20.3	11.8	11.5	11.4	11.6	8.8	8.4	0.8	0.8	0.0	0.0	0.2	0.3
	4	suc	1	18.4	18.0	18.2	18.2	9.7	9.6	9.8	9.7	8.5	8.4	0.9	1.8	-0.9	0.7		
			2	17.8	17.7	17.6	17.7	10.9	10.7	11.0	10.9	6.8	8.4	2.9	1.8	1.1	1.3		
			3	16.5	16.8	16.9	16.7	8.7	9.0	9.1	8.9	7.8	8.4	1.5	1.8	-0.3	0.1	2.1	1.0
		glc	1	17.6	17.6	17.8	17.7	10.1	9.8	10.0	10.0	7.7	8.4	1.6	1.0	0.6	0.3		
			2	17.8	17.9	18.0	17.9	10.1	10.0	9.9	10.0	7.9	8.4	1.4	1.0	0.4	0.1		

			3	19.8	19.6	19.7	**19.7**	10.1	1.1	10.3	**7.2**	12.5	8.4	0.1	**1.0**	-1.0	0.9	1.4	**0.8**
		fru	1	20.5	21.0	20.7	**20.7**	12.5	12.4	13.0	**12.6**	8.1	8.4	1.2	**0.8**	0.4	0.2		
			2	19.1	19.7	19.6	**19.5**	10.0	10.2	10.3	**10.2**	9.3	8.4	0.5	**0.8**	-0.2	0.1		
			3	21.0	21.5	21.4	**21.3**	12.0	12.1	12.1	**12.1**	9.2	8.4	0.5	**0.8**	-0.2	0.0	0.3	**0.4**
	7	suc	1	25.0	25.0	25.3	**25.1**	16.7	16.8	16.7	**16.7**	8.4	8.4	1.0	**1.1**	-0.1	0.0		
			2	23.6	23.7	24.0	**23.8**	16.1	16.1	16.0	**16.1**	7.7	8.4	1.6	**1.1**	0.5	0.2		
			3	27.4	27.0	27.4	**27.3**	18.5	18.3	18.6	**18.5**	8.8	8.4	0.7	**1.1**	-0.4	0.1	0.4	**0.4**
		glc	1	23.7	23.9	23.2	**23.6**	14.9	15.1	15.2	**15.1**	8.5	8.4	0.9	**1.0**	-0.1	0.0		
			2	21.8	21.8	21.3	**21.6**	14.0	13.8	13.8	**13.9**	7.8	8.4	1.5	**1.0**	0.5	0.2		
			3	29.3	28.8	28.4	**28.8**	19.9	19.9	19.8	**19.9**	9.0	8.4	0.7	**1.0**	-0.4	0.1	0.4	**0.4**
		fru	1	23.0	23.0	22.7	**22.9**	14.1	14.2	14.1	**14.1**	8.8	8.4	0.8	**0.9**	-0.1	0.0		
			2	22.8	22.8	22.5	**22.7**	14.2	14.3	14.8	**14.4**	8.3	8.4	1.1	**0.9**	0.2	0.0		
			3	23.1	23.1	22.8	**23.0**	14.5	14.4	14.4	**14.4**	8.6	8.4	0.9	**0.9**	0.0	0.0	0.1	**0.2**
	14	suc	1	23.9	24.0	24.0	**24.0**	15.7	15.7	15.6	**15.7**	8.3	8.4	1.0	**1.2**	-0.2	0.0		
			2	22.2	22.1	22.0	**22.1**	14.6	14.6	14.6	**14.6**	7.5	8.4	1.8	**1.2**	0.6	0.4		
			3	23.7	23.7	23.6	**23.7**	14.9	14.9	15.2	**15.0**	8.7	8.4	0.8	**1.2**	-0.4	0.2	0.6	**0.5**
		glc	1	22.0	22.0	22.1	**22.0**	14.2	14.6	14.2	**14.3**	7.7	8.4	1.6	**1.0**	0.6	0.4		
			2	23.7	23.8	23.8	**23.8**	14.9	14.9	14.8	**14.9**	8.9	8.4	0.7	**1.0**	-0.3	0.1		
			3	21.8	22.0	21.9	**21.9**	12.8	12.8	12.9	**12.8**	9.1	8.4	0.6	**1.0**	-0.3	0.1	0.6	**0.5**
		fru	1	21.8	21.7	21.8	**21.8**	14.0	14.1	14.3	**14.1**	7.6	8.4	1.7	**0.8**	0.8	0.7		
			2	23.6	23.7	23.7	**23.7**	14.7	14.6	14.6	**14.6**	9.0	8.4	0.6	**0.8**	-0.2	0.0		
			3	25.9	26.1	25.4	**25.8**	15.1	15.3	15.3	**15.2**	10.6	8.4	0.2	**0.8**	-0.6	0.4	1.1	**0.7**

Gene	Day	Treatment	Sample	CT 1	CT 2	CT 3	Avg CT	18S CT 1	18S CT 2	18S CT3	Avg 18S CT	ΔCT	Avg D0 ΔCT	Fold Change	Avg Fold Change	Deviation	$Deviation^2$	$\Sigma Deviation^2$	SD
DXR	0	none	1	31.4	31.3	31.9	**31.5**	19.8	19.7	20.0	**19.8**	11.7	11.0	0.6	**1.1**	-0.4	0.2		
			2	32.1	32.0	32.3	**32.1**	21.4	21.9	21.1	**21.5**	10.7	11.0	1.3	**1.1**	0.2	0.0		
			3	30.9	30.4	31.1	**30.8**	20.1	20.3	20.2	**20.2**	10.6	11.0	1.3	**1.1**	0.3	0.1	0.3	**0.4**
	1	suc	1	32.3	32.9	32.3	**32.5**	22.1	21.9	22.1	**22.0**	10.5	11.0	1.4	**1.0**	0.4	0.2		
			2	34.3	34.5	34.2	**34.3**	23.3	23.5	23.2	**23.3**	11.0	11.0	1.0	**1.0**	0.0	0.0		
			3	33.1	32.5	32.4	**32.7**	20.8	20.9	20.9	**20.9**	11.8	11.0	0.6	**1.0**	-0.4	0.2	0.4	**0.4**
		glc	1	33.1	33.4	33.0	**33.2**	25.0	25.6	24.8	**25.1**	8.0	11.0	7.8	**7.4**	0.3	0.1		
			2	32.9	33.0	33.5	**33.1**	25.5	25.1	25.8	**25.5**	7.7	11.0	10.0	**7.4**	2.6	6.6		
			3	32.8	33.1	33.1	**33.0**	24.0	24.1	24.5	**24.2**	8.8	11.0	4.6	**7.4**	-2.9	8.3	15.0	**2.7**
		fru	1	30.9	31.5	30.7	**31.0**	20.4	20.0	20.4	**20.3**	10.8	11.0	1.2	**2.4**	-1.2	1.5		
			2	33.0	32.1	33.1	**32.7**	23.4	23.4	23.7	**23.5**	9.2	11.0	3.4	**2.4**	1.0	1.0		
			3	32.0	32.6	32.3	**32.3**	22.6	22.8	22.7	**22.7**	9.6	11.0	2.6	**2.4**	0.2	0.1	2.5	**1.1**
	2	suc	1	31.7	31.8	32.0	**31.8**	21.7	21.0	21.6	**21.4**	10.4	11.0	1.5	**1.9**	-0.4	0.2		
			2	30.7	31.1	30.7	**30.8**	21.1	20.9	21.6	**21.2**	9.6	11.0	2.6	**1.9**	0.7	0.4		
			3	32.8	33.2	32.7	**32.9**	22.2	23.0	22.6	**22.6**	10.3	11.0	1.6	**1.9**	-0.3	0.1	0.7	**0.6**
		glc	1	30.2	30.5	30.8	**30.5**	21.1	21.4	20.9	**21.1**	9.4	11.0	3.1	**3.6**	-0.5	0.3		
			2	28.5	28.7	28.7	**28.6**	19.5	19.6	19.6	**19.6**	9.1	11.0	3.8	**3.6**	0.2	0.0		
			3	33.2	33.9	33.0	**33.4**	24.4	24.2	24.5	**24.4**	9.0	11.0	4.0	**3.6**	0.4	0.1	0.4	**0.5**
		fru	1	28.3	28.1	28.1	**28.2**	18.6	18.4	18.7	**18.6**	9.6	11.0	2.6	**1.7**	0.9	0.9		
			2	29.7	30.1	30.0	**29.9**	19.7	19.7	19.7	**19.7**	10.2	11.0	1.7	**1.7**	0.0	0.0		
			3	30.0	30.1	30.9	**30.3**	18.9	18.9	19.1	**19.0**	11.4	11.0	0.8	**1.7**	-0.9	0.9	1.7	**0.9**
	3	suc	1	20.2	20.4	20.5	**20.4**	8.9	9.2	9.5	**9.2**	11.2	11.0	0.9	**2.2**	-1.4	1.8		
			2	19.6	19.4	19.3	**19.4**	10.0	10.6	10.4	**10.3**	9.1	11.0	3.7	**2.2**	1.5	2.1		
			3	21.7	21.6	21.8	**21.7**	11.7	11.8	11.9	**11.8**	9.9	11.0	2.1	**2.2**	-0.1	0.0	4.0	**1.4**
		glc	1	24.7	24.2	24.6	**24.5**	16.4	16.2	16.4	**16.3**	8.2	11.0	7.1	**5.8**	1.3	1.7		
			2	25.4	25.5	25.9	**25.6**	16.1	16.1	16.2	**16.1**	9.5	11.0	2.9	**5.8**	-2.9	8.5		
			3	23.9	23.6	23.6	**23.7**	15.0	15.9	15.9	**15.6**	8.1	11.0	7.4	**5.8**	1.6	2.6	12.8	**2.5**
		fru	1	23.3	23.4	23.5	**23.4**	13.4	13.3	13.3	**13.3**	10.1	11.0	1.9	**1.5**	0.4	0.1		
			2	24.2	24.3	24.4	**24.3**	13.8	13.9	13.8	**13.8**	10.5	11.0	1.4	**1.5**	-0.1	0.0		

	4	suc	1	22.0	21.8	21.7	**21.8**	11.3	11.0	10.9	**11.1**	10.8	11.0	1.2	**2.5**	-1.3	1.8		
			2	22.1	21.7	21.6	**21.8**	12.6	12.4	12.8	**12.6**	9.2	11.0	3.5	**2.5**	1.0	0.9		
			3	19.5	19.5	19.5	**19.5**	10.0	10.0	10.1	**10.0**	9.5	11.0	2.9	**2.5**	0.4	0.1	2.8	**1.2**
		glc	1	21.7	21.8	21.8	**21.8**	13.0	12.9	13.2	**13.0**	8.7	11.0	4.8	**3.1**	1.6	2.6		
			2	22.4	22.6	22.7	**22.6**	12.9	12.6	12.7	**12.7**	9.8	11.0	2.2	**3.1**	-0.9	0.8		
			3	22.4	22.2	22.4	**22.3**	12.2	12.8	12.9	**12.6**	9.7	11.0	2.4	**3.1**	-0.7	0.5	4.0	**1.4**
		fru	1	21.9	22.0	21.8	**21.9**	11.3	11.4	11.4	**11.4**	10.5	11.0	1.4	**1.1**	0.3	0.1		
			2	21.1	21.7	21.2	**21.3**	10.8	10.2	10.2	**10.4**	10.9	11.0	1.0	**1.1**	0.0	0.0		
			3	25.2	25.2	25.6	**25.3**	14.0	13.9	14.1	**14.0**	11.3	11.0	0.8	**1.1**	-0.3	0.1	0.2	**0.3**
	7	suc	1	24.6	24.3	24.1	**24.3**	14.0	14.1	13.9	**14.0**	10.3	11.0	1.6	**2.1**	-0.5	0.2		
			2	23.5	23.6	23.7	**23.6**	13.9	14.0	14.1	**14.0**	9.6	11.0	2.6	**2.1**	0.6	0.3		
			3	26.5	26.4	26.9	**26.6**	16.3	16.8	16.7	**16.6**	10.0	11.0	2.0	**2.1**	-0.1	0.0	0.6	**0.5**
		glc	1	23.5	23.8	23.7	**23.7**	13.5	13.6	13.9	**13.7**	10.0	11.0	2.0	**3.1**	-1.1	1.3		
			2	22.4	22.6	22.3	**22.4**	14.0	14.0	13.9	**14.0**	8.5	11.0	5.7	**3.1**	2.6	6.9		
			3	27.9	27.2	27.6	**27.6**	17.2	17.2	17.4	**17.3**	10.3	11.0	1.6	**3.1**	-1.5	2.3	10.5	**2.3**
		fru	1	23.6	23.7	23.9	**23.7**	13.7	13.8	13.8	**13.8**	10.0	11.0	2.0	**1.7**	0.4	0.1		
			2	24.5	24.7	24.8	**24.7**	14.2	14.4	14.7	**14.4**	10.2	11.0	1.7	**1.7**	0.0	0.0		
			3	25.6	25.7	25.1	**25.5**	14.7	14.8	15.0	**14.8**	10.6	11.0	1.3	**1.7**	-0.4	0.1	0.3	**0.4**
	14	suc	1	24.7	24.3	24.4	**24.5**	13.9	13.9	13.8	**13.9**	10.6	11.0	1.3	**1.7**	-0.4	0.2		
			2	23.1	23.0	22.9	**23.0**	13.3	13.1	13.2	**13.2**	9.8	11.0	2.3	**1.7**	0.6	0.3		
			3	24.7	24.4	24.7	**24.6**	14.3	14.3	14.1	**14.2**	10.4	11.0	1.5	**1.7**	-0.2	0.0	0.5	**0.5**
		glc	1	22.7	22.7	23.0	**22.8**	13.5	13.7	13.9	**13.7**	9.1	11.0	3.7	**3.4**	0.3	0.1		
			2	22.3	21.9	22.3	**22.2**	13.6	13.4	13.4	**13.5**	8.7	11.0	4.9	**3.4**	1.5	2.2		
			3	22.8	23.0	22.8	**22.9**	12.5	12.6	12.5	**12.5**	10.3	11.0	1.6	**3.4**	-1.8	3.3	5.6	**1.7**
		fru	1	23.3	23.2	23.2	**23.2**	13.5	13.5	13.2	**13.4**	9.8	11.0	2.2	**2.0**	0.2	0.0		
			2	23.7	23.8	23.8	**23.8**	13.9	14.2	14.1	**14.1**	9.7	11.0	2.4	**2.0**	0.4	0.2		
			3	22.8	22.9	22.6	**22.8**	12.8	12.2	12.0	**12.3**	10.4	11.0	1.5	**2.0**	-0.6	0.3	0.5	**0.5**
Gene	**Day**	**Treatment**	**Sample**	**CT 1**	**CT 2**	**CT 3**	**Avg CT**	**18S CT 1**	**18S CT 2**	**18S CT3**	**Avg 18S CT**	**ΔCT**	**Avg D0 ΔCT**	**Fold Change**	**Avg Fold Change**	**Deviation**	**Deviation^2**	**ΣDeviation^2**	**SD**
DXS	0	none	1	31.4	31.5	31.8	**31.6**	19.8	19.7	20.0	**19.8**	11.7	11.0	0.6	**1.1**	-0.5	0.2		
			2	32.5	32.4	32.5	**32.5**	21.4	21.9	21.1	**21.5**	11.0	11.0	1.0	**1.1**	-0.1	0.0		
			3	30.9	30.4	30.1	**30.5**	20.1	20.3	20.2	**20.2**	10.3	11.0	1.7	**1.1**	0.6	0.3	0.6	**0.5**
	1	suc	1	32.1	33.1	32.7	**32.6**	22.1	21.9	22.1	**22.0**	10.6	11.0	1.3	**1.1**	0.3	0.1		
			2	34.2	33.6	34.8	**34.2**	23.3	23.5	23.2	**23.3**	10.9	11.0	1.1	**1.1**	0.0	0.0		
			3	31.8	33.1	32.0	**32.3**	20.8	20.9	20.9	**20.9**	11.4	11.0	0.7	**1.1**	-0.3	0.1	0.2	**0.3**
		glc	1	35.3	33.1	34.0	**34.1**	25.0	25.6	24.8	**25.1**	9.0	11.0	4.0	**5.4**	-1.4	1.9		
			2	34.9	34.1	33.2	**34.1**	25.5	25.1	25.8	**25.5**	8.6	11.0	5.3	**5.4**	-0.1	0.0		
			3	32.6	32.3	32.4	**32.4**	24.0	24.1	24.5	**24.2**	8.2	11.0	6.8	**5.4**	1.4	2.1	3.9	**1.4**
		fru	1	31.1	30.3	29.9	**30.4**	20.4	20.0	20.4	**20.3**	10.2	11.0	1.8	**1.8**	0.0	0.0		
			2	34.8	32.7	35.0	**34.2**	23.4	23.4	23.7	**23.5**	10.7	11.0	1.3	**1.8**	-0.5	0.3		
			3	32.2	32.5	32.8	**32.5**	22.6	22.8	22.7	**22.7**	9.8	11.0	2.3	**1.8**	0.5	0.3	0.5	**0.5**
	2	suc	1	32.4	32.0	33.1	**32.5**	21.7	21.0	21.6	**21.4**	11.1	11.0	1.0	**1.4**	-0.4	0.2		
			2	31.8	30.9	32.0	**31.6**	21.1	20.9	21.6	**21.2**	10.4	11.0	1.6	**1.4**	0.2	0.0		
			3	33.2	33.0	32.7	**33.0**	22.2	23.0	22.6	**22.6**	10.4	11.0	1.6	**1.4**	0.2	0.0	0.2	**0.3**
		glc	1	29.4	29.8	29.4	**29.5**	21.1	21.4	20.9	**21.1**	8.4	11.0	6.1	**11.0**	-5.0	24.8		
			2	26.4	27.2	27.3	**27.0**	19.5	19.6	19.6	**19.6**	7.4	11.0	12.1	**11.0**	1.1	1.2		
			3	31.3	31.5	31.6	**31.5**	24.4	24.2	24.5	**24.4**	7.1	11.0	14.9	**11.0**	3.9	15.1	41.1	**4.5**
		fru	1	29.7	29.9	30.0	**29.9**	18.6	18.4	18.7	**18.6**	11.3	11.0	0.8	**1.4**	-0.5	0.3		
			2	29.8	29.6	30.2	**29.9**	19.7	19.7	19.7	**19.7**	10.2	11.0	1.8	**1.4**	0.4	0.2		
			3	29.0	29.6	29.6	**29.4**	18.9	18.9	19.1	**19.0**	10.4	11.0	1.5	**1.4**	0.1	0.0	0.5	**0.5**
	3	suc	1	19.2	19.8	19.5	**19.5**	8.9	9.2	9.5	**9.2**	10.3	11.0	1.6	**1.8**	-0.2	0.0		
			2	20.3	20.1	20.2	**20.2**	10.0	10.6	10.4	**10.3**	9.9	11.0	2.2	**1.8**	0.4	0.1		
			3	22.1	22.7	21.3	**22.0**	11.7	11.8	11.9	**11.8**	10.2	11.0	1.7	**1.8**	-0.1	0.0	0.2	**0.3**

			2	25.7	25.9	25.5	**25.7**	16.1	16.1	16.2	**16.1**	9.6	11.0	2.7	**4.8**	-2.1	4.5		
			3	24.2	24.0	24.2	**24.1**	15.0	15.9	15.9	**15.6**	8.5	11.0	5.5	**4.8**	0.7	0.5	6.9	**1.9**
		fru	1	23.5	23.1	23.1	**23.2**	13.4	13.3	13.3	**13.3**	9.9	11.0	2.1	**1.7**	0.4	0.2		
			2	23.6	24.7	24.3	**24.2**	13.8	13.9	13.8	**13.8**	10.4	11.0	1.6	**1.7**	-0.2	0.0		
			3	23.8	23.9	24.0	**23.9**	13.1	13.6	13.6	**13.4**	10.5	11.0	1.4	**1.7**	-0.3	0.1	0.3	**0.4**
	4	suc	1	22.2	22.0	22.3	**22.2**	11.3	11.0	10.9	**11.1**	11.1	11.0	0.9	**1.7**	-0.7	0.5		
			2	22.7	22.4	22.3	**22.5**	12.6	12.4	12.8	**12.6**	9.9	11.0	2.2	**1.7**	0.5	0.3		
			3	20.7	19.8	20.0	**20.2**	10.0	10.0	10.1	**10.0**	10.1	11.0	1.8	**1.7**	0.2	0.0	0.8	**0.6**
		glc	1	22.6	22.7	22.2	**22.5**	13.0	12.9	13.2	**13.0**	9.5	11.0	2.9	**1.9**	1.0	1.0		
			2	22.3	22.5	22.3	**22.4**	12.9	12.6	12.7	**12.7**	9.6	11.0	2.6	**1.9**	0.7	0.4		
			3	25.6	25.4	25.4	**25.5**	12.2	12.8	12.9	**12.6**	12.8	11.0	0.3	**1.9**	-1.6	2.7	4.1	**1.4**
		fru	1	22.0	21.9	21.8	**21.9**	11.3	11.4	11.4	**11.4**	10.5	11.0	1.4	**0.8**	0.5	0.3		
			2	21.5	21.8	21.5	**21.6**	10.8	10.2	10.2	**10.4**	11.2	11.0	0.9	**0.8**	0.0	0.0		
			3	26.8	26.8	26.7	**26.8**	14.0	13.9	14.1	**14.0**	12.8	11.0	0.3	**0.8**	-0.6	0.3	0.6	**0.5**
	7	suc	1	24.8	24.6	24.6	**24.7**	14.0	14.1	13.9	**14.0**	10.7	11.0	1.3	**1.6**	-0.3	0.1		
			2	23.9	24.0	24.1	**24.0**	13.9	14.0	14.1	**14.0**	10.0	11.0	2.0	**1.6**	0.4	0.2		
			3	26.9	27.2	27.1	**27.1**	16.3	16.8	16.7	**16.6**	10.5	11.0	1.4	**1.6**	-0.1	0.0	0.3	**0.4**
		glc	1	24.6	24.7	24.5	**24.6**	13.5	13.6	13.9	**13.7**	10.9	11.0	1.0	**1.7**	-0.6	0.4		
			2	23.4	23.2	23.3	**23.3**	14.0	14.0	13.9	**14.0**	9.3	11.0	3.2	**1.7**	1.5	2.3		
			3	28.6	28.7	28.7	**28.7**	17.2	17.2	17.4	**17.3**	11.4	11.0	0.8	**1.7**	-0.9	0.8	3.5	**1.3**
		fru	1	24.2	24.3	24.3	**24.3**	13.7	13.8	13.8	**13.8**	10.5	11.0	1.4	**1.2**	0.2	0.0		
			2	24.9	25.2	25.0	**25.0**	14.2	14.4	14.7	**14.4**	10.6	11.0	1.3	**1.2**	0.1	0.0		
			3	25.7	25.8	26.0	**25.8**	14.7	14.8	15.0	**14.8**	11.0	11.0	1.0	**1.2**	-0.2	0.1	0.1	**0.2**
	14	suc	1	24.4	24.5	24.1	**24.3**	13.9	13.9	13.8	**13.9**	10.5	11.0	1.4	**1.9**	-0.4	0.2		
			2	23.3	23.2	23.1	**23.2**	13.3	13.1	13.2	**13.2**	10.0	11.0	2.0	**1.9**	0.1	0.0		
			3	24.0	24.2	24.2	**24.1**	14.3	14.3	14.1	**14.2**	9.9	11.0	2.1	**1.9**	0.3	0.1	0.3	**0.4**
		glc	1	22.8	23.1	23.0	**23.0**	13.5	13.7	13.9	**13.7**	9.3	11.0	3.3	**3.1**	0.2	0.1		
			2	22.7	22.4	22.3	**22.5**	13.6	13.4	13.4	**13.5**	9.0	11.0	4.0	**3.1**	0.9	0.8		
			3	22.6	22.8	22.4	**22.6**	12.5	12.6	12.5	**12.5**	10.1	11.0	1.9	**3.1**	-1.2	1.4	2.3	**1.1**
		fru	1	23.6	23.9	23.8	**23.8**	13.5	13.5	13.2	**13.4**	10.4	11.0	1.6	**1.2**	0.3	0.1		
			2	24.6	25.1	25.2	**25.0**	13.9	14.2	14.1	**14.1**	10.9	11.0	1.1	**1.2**	-0.2	0.0		
			3	23.3	23.5	23.0	**23.3**	12.8	12.2	12.0	**12.3**	10.9	11.0	1.0	**1.2**	-0.2	0.0	0.2	**0.3**

Gene	Day	Treatment	Sample	CT 1	CT 2	CT 3	Avg CT	18S CT 1	18S CT 2	18S CT3	Avg 18S CT	ΔCT	Avg D0 ΔCT	Fold Change	Avg Fold Change	Deviation	$Deviation^2$	$\Sigma Deviation^2$	SD
SQS	0	none	1	27.8	27.8	28.3	**28.0**	16.4	16.4	16.4	**16.4**	11.6	11.4	0.9	**1.0**	-0.1	0.0		
			2	29.0	29.3	29.7	**29.3**	18.4	18.3	18.1	**18.3**	11.1	11.4	1.3	**1.0**	0.3	0.1		
			3	28.0	27.8	28.4	**28.1**	16.4	16.3	16.6	**16.4**	11.6	11.4	0.9	**1.0**	-0.2	0.0	0.1	**0.2**
	1	suc	1	31.0	31.2	31.3	**31.2**	19.3	19.1	19.4	**19.3**	11.9	11.4	0.7	**1.4**	-0.7	0.5		
			2	29.4	29.5	29.4	**29.4**	19.6	19.8	19.8	**19.7**	9.7	11.4	3.3	**1.4**	1.9	3.5		
			3	30.6	31.3	31.0	**31.0**	17.6	17.6	17.7	**17.6**	13.3	11.4	0.3	**1.4**	-1.2	1.4	5.4	**1.6**
		glc	1	26.9	27.7	27.2	**27.3**	17.8	17.8	17.8	**17.8**	9.5	11.4	3.9	**3.1**	0.8	0.7		
			2	29.0	28.9	29.0	**29.0**	19.0	19.0	18.9	**19.0**	10.0	11.4	2.7	**3.1**	-0.4	0.1		
			3	28.9	29.0	28.9	**28.9**	18.8	19.0	18.9	**18.9**	10.0	11.4	2.6	**3.1**	-0.4	0.2	1.0	**0.7**
		fru	1	28.9	29.3	29.1	**29.1**	16.7	16.7	16.7	**16.7**	12.4	11.4	0.5	**0.6**	-0.1	0.0		
			2	29.1	29.5	29.3	**29.3**	17.2	17.3	17.2	**17.2**	12.1	11.4	0.6	**0.6**	0.1	0.0		
			3	27.9	28.3	28.1	**28.1**	16.0	15.9	15.4	**15.8**	12.3	11.4	0.5	**0.6**	0.0	0.0	0.0	**0.1**
	2	suc	1	28.5	28.3	28.4	**28.4**	18.6	18.4	18.6	**18.5**	9.9	11.4	2.9	**2.6**	0.3	0.1		
			2	28.1	27.5	27.8	**27.8**	17.7	17.6	17.5	**17.6**	10.2	11.4	2.3	**2.6**	-0.3	0.1		
			3	26.6	26.6	26.6	**26.6**	16.6	16.5	16.4	**16.5**	10.1	11.4	2.5	**2.6**	-0.1	0.0	0.2	**0.3**
		glc	1	26.2	26.3	26.3	**26.3**	16.5	16.6	16.4	**16.5**	9.8	11.4	3.2	**2.5**	0.6	0.4		
			2	26.0	26.6	26.3	**26.3**	15.6	15.4	15.7	**15.6**	10.7	11.4	1.6	**2.5**	-0.9	0.8		
			3	27.8	27.9	27.8	**27.8**	17.7	18.1	17.8	**17.9**	10.0	11.4	2.7	**2.5**	0.2	0.1	1.3	**0.8**
		fru	1	26.4	27.0	26.7	**26.7**	15.6	15.6	15.5	**15.6**	11.1	11.4	1.2	**3.8**	-2.6	6.6		

		3	26.6	26.7	26.7	**26.7**	16.8	16.7	16.9	**16.8**	9.9	11.4	2.9	**3.8**	-0.9	0.7	19.2	**3.1**
3	suc	1	32.1	31.9	32.3	**32.1**	21.2	21.4	21.8	**21.5**	10.6	11.4	1.7	**2.8**	-1.1	1.2		
		2	23.6	23.7	23.7	**23.7**	12.9	12.7	13.1	**12.9**	10.8	11.4	1.6	**2.8**	-1.2	1.5		
		3	25.6	25.1	25.4	**25.4**	16.3	16.3	16.3	**16.3**	9.1	11.4	5.1	**2.8**	2.3	5.3	8.0	**2.0**
	glc	1	30.8	30.8	31.3	**31.0**	18.8	18.9	18.8	**18.8**	12.1	11.4	0.6	**1.0**	-0.4	0.2		
		2	32.4	32.6	32.9	**32.6**	21.6	22.0	21.5	**21.7**	10.9	11.4	1.4	**1.0**	0.4	0.1		
		3	31.7	31.7	31.1	**31.5**	20.1	19.9	20.6	**20.2**	11.3	11.4	1.1	**1.0**	0.1	0.0	0.3	**0.4**
	fru	1	27.7	27.8	27.9	**27.8**	17.0	16.9	17.1	**17.0**	10.8	11.4	1.5	**0.9**	0.7	0.5		
		2	31.6	32.2	31.9	**31.9**	18.9	18.8	18.9	**18.9**	13.0	11.4	0.3	**0.9**	-0.5	0.3		
		3	28.9	28.9	30.0	**29.3**	17.5	17.6	17.1	**17.4**	11.9	11.4	0.7	**0.9**	-0.1	0.0	0.8	**0.6**
4	suc	1	27.4	27.4	27.6	**27.5**	16.4	16.5	16.4	**16.4**	11.0	11.4	1.3	**5.3**	-4.0	16.0		
		2	32.0	31.1	32.6	**31.9**	23.4	23.6	23.6	**23.5**	8.4	11.4	8.3	**5.3**	3.0	9.0		
		3	24.0	24.4	24.4	**24.3**	15.4	15.3	15.8	**15.5**	8.8	11.4	6.3	**5.3**	1.0	1.0	26.0	**3.6**
	glc	1	24.7	24.8	24.7	**24.7**	16.1	16.0	16.3	**16.1**	8.6	11.4	7.1	**4.3**	2.8	7.9		
		2	25.7	25.6	25.5	**25.6**	15.8	15.6	16.0	**15.8**	9.8	11.4	3.1	**4.3**	-1.2	1.4		
		3	25.8	25.8	26.0	**25.9**	15.9	15.7	15.9	**15.8**	10.0	11.4	2.6	**4.3**	-1.6	2.7	12.0	**2.4**
	fru	1	29.9	29.6	30.5	**30.0**	20.3	20.4	20.9	**20.5**	9.5	11.4	3.9	**3.9**	0.0	0.0		
		2	28.5	28.4	28.1	**28.3**	19.0	18.5	18.8	**18.8**	9.6	11.4	3.6	**3.9**	-0.2	0.1		
		3	28.0	28.0	28.0	**28.0**	18.5	18.7	18.6	**18.6**	9.4	11.4	4.1	**3.9**	0.2	0.0	0.1	**0.2**
7	suc	1	31.3	31.4	31.4	**31.4**	19.7	19.9	19.8	**19.8**	11.6	11.4	0.9	**1.1**	-0.2	0.0		
		2	28.9	29.0	29.0	**29.0**	17.9	17.8	18.0	**17.9**	11.1	11.4	1.3	**1.1**	0.2	0.0		
		3	32.6	32.4	33.4	**32.8**	21.5	21.2	21.6	**21.4**	11.4	11.4	1.0	**1.1**	0.0	0.0	0.1	**0.2**
	glc	1	31.0	31.2	30.8	**31.0**	20.8	20.6	20.7	**20.7**	10.3	11.4	2.2	**1.9**	0.3	0.1		
		2	29.7	29.6	29.6	**29.6**	19.5	19.4	19.4	**19.4**	10.2	11.4	2.3	**1.9**	0.4	0.2		
		3	30.8	30.0	31.1	**30.6**	19.4	19.5	19.4	**19.4**	11.2	11.4	1.2	**1.9**	-0.7	0.5	0.8	**0.6**
	fru	1	29.8	29.9	29.9	**29.9**	19.1	19.1	19.1	**19.1**	10.8	11.4	1.6	**1.4**	0.2	0.0		
		2	29.0	29.5	29.7	**29.4**	18.2	18.3	18.4	**18.3**	11.1	11.4	1.3	**1.4**	-0.1	0.0		
		3	29.9	30.3	30.1	**30.1**	19.1	19.1	19.1	**19.1**	11.0	11.4	1.3	**1.4**	0.0	0.0	0.1	**0.2**
14	suc	1	29.5	29.8	29.1	**29.5**	20.3	20.2	19.8	**20.1**	9.4	11.4	4.2	**5.4**	-1.2	1.5		
		2	28.7	28.8	29.1	**28.9**	17.5	17.4	18.5	**17.8**	11.1	11.4	1.3	**5.4**	-4.1	16.9		
		3	28.6	29.0	29.1	**28.9**	20.5	21.2	21.0	**20.9**	8.0	11.4	10.7	**5.4**	5.3	28.5	46.8	**4.8**
	glc	1	29.1	29.1	29.1	**29.1**	21.1	21.6	21.4	**21.4**	7.7	11.4	12.9	**27.3**	-14.4	207.0		
		2	26.1	26.1	26.1	**26.1**	20.0	20.0	20.0	**20.0**	6.1	11.4	40.0	**27.3**	12.7	161.9		
		3	26.3	26.4	26.4	**26.4**	19.7	20.0	19.7	**19.8**	6.6	11.4	29.0	**27.3**	1.7	2.8	371.7	**13.6**
	fru	1	28.4	28.6	29.3	**28.8**	19.5	20.0	20.2	**19.9**	8.9	11.4	5.9	**2.8**	3.0	9.2		
		2	29.2	29.3	29.6	**29.4**	18.7	18.7	19.3	**18.9**	10.5	11.4	1.9	**2.8**	-0.9	0.8		

Table A4. Statistical significance in gene expression in glucose and fructose treatments compared to sucrose treatment.

Gene	Day	Treatment	P-value
ADS	1	Glc	0.0495
		Fru	0.0495
	2	Glc	0.8273
		Fru	0.0495
	3	Glc	0.0495
		Fru	0.0495
	4	Glc	0.0495
		Fru	0.0495
	7	Glc	0.0495
		Fru	0.0495
	14	Glc	0.0495
		Fru	0.2752
CYP	1	Glc	0.0495
		Fru	0.0809
	2	Glc	0.0495
		Fru	0.5127
	3	Glc	0.0495
		Fru	0.0495
	4	Glc	0.8273
		Fru	0.0495
	7	Glc	0.0495
		Fru	0.6625
	14	Glc	0.3827
		Fru	0.1904
FPS	1	Glc	0.0495
		Fru	0.0495
	2	Glc	0.0495
		Fru	0.5126
	3	Glc	0.2752
		Fru	0.2752
	4	Glc	0.2752
		Fru	0.2752
	7	Glc	0.1904
		Fru	0.8272
	14	Glc	0.2752
		Fru	0.2752
HMGR	1	Glc	0.0495
		Fru	0.0495
	2	Glc	0.1266
		Fru	0.0495
	3	Glc	0.8272
		Fru	0.1266
	4	Glc	0.6626
		Fru	0.1266
	7	Glc	0.5126
		Fru	0.6626
	14	Glc	0.2752
		Fru	0.2752

DXR	1	Glc	0.0495
		Fru	0.1266
	2	Glc	0.0495
		Fru	0.8272
	3	Glc	0.1266
		Fru	0.5126
	4	Glc	0.8272
		Fru	0.1904
	7	Glc	0.8272
		Fru	0.2752
	14	Glc	0.1904
		Fru	0.3828
DXS	1	Glc	0.0495
		Fru	0.1266
	2	Glc	0.0495
		Fru	0.8273
	3	Glc	0.0495
		Fru	0.6625
	4	Glc	0.5127
		Fru	0.1904
	7	Glc	0.5127
		Fru	0.5127
	14	Glc	0.2752
		Fru	0.0809
SQS	1	Glc	0.2752
		Fru	0.5127
	2	Glc	0.6625
		Fru	0.6625
	3	Glc	0.0495
		Fru	0.0495
	4	Glc	0.8273
		Fru	0.5127
	7	Glc	0.1266
		Fru	0.0809
	14	Glc	0.0495
		Fru	0.5127

Table A5. Statistical significance in gene expression in budding and flowering plants compared to vegetative plants

Gene	Dev. Stage	P-value
ADS	Budding	0.0045
	Flowering	0.0708
CYP	Budding	0.0045
	Flowering	0.1636
FPS	Budding	0.0045
	Flowering	0.0072
HMGR	Budding	0.0045
	Flowering	0.0127
DXR	Budding	0.1481
	Flowering	0.0045
DXS	Budding	0.0557
	Flowering	0.0045
SQS	Budding	0.0873
	Flowering	0.0301